Evolution of reproductive isolation in sympatric Arctic charr morphs (*Salvelinus alpinus*)

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Faculty of Life and Environmental Sciences
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2022

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Dissertation submitted in partial fulfillment of a
Philosophiae Doctor degree in Biology

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Reykjavik, May 2022

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Bibliographic information:
Quentin Jean-Baptiste Horta-Lacueva, 2022, Evolution of reproductive isolation in sympatric Arctic charr morphs , PhD dissertation, Faculty of Life and Environmental Sciences, University of Iceland, XX pp.

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ISBN 978-9935-9647-5-5

Printing: Háskólaprent
Reykjavik, Iceland, May 2022

Abstract

The theory of divergence by trophic polymorphism, an important part of diversification in vertebrates, has recently been extended to encompass the interplay of developmental, ecological and evolutionary processes (Eco-Evo-Devo dynamics). However, this extended theory doesn't thoroughly explain the evolution of reproductive isolation, which is unfortunate considering the recent advances from the field of speciation. In this thesis, I argue that the Arctic charr morphs of Thingvallavatn are an ideal system to study how reproductive isolation is embedded within the theory of divergence by resource polymorphism, which I present through five papers. First, I focused on two sympatric morphs, the small-benthic (SB) and the planktivorous (PL) charr. Common-garden experiments showed limited evidence for hybridization to affect the structure of trait covariance in both morphs, regarding morphology, developmental timing and feeding behaviour (Paper I), and personality traits (Paper II). However, information on gene expression variability in embryos indicated that hybridization might influence evolvability (Paper III). Multiple reproductive barriers between the two morphs, involving habitat use, assortative mating and hybrid development were also assessed (Paper IV). Finally, Paper V combines field studies and rearing experiments to explore the interplay between habitat choice and offspring development in the large-benthic (LB) charr, which spawns earlier in the season than the other morphs. The results suggested that LB-charr favour temperature conditions that may delay offspring development. Altogether, these findings provide an overview on reproductive isolation among the Arctic charr morphs of Thingvallavatn and constitute a primer to study speciation in an Eco-Evo-Devo context.

Útdráttur

Fjölbrigðni tengd fæðu- og búsvæðavali er talin geta verið mikilvæg í ferli afbrigða- og tegundamyndunar meðal hryggdýra. Nýlega hafa komið fram heildstæðari hugmyndir um þessi ferli sem taka til samspils þroskunar-, vistfræði- og þróunarferla, en bagalegt er að þær sniðganga mikilvægi þróunar æxlunareinangrunar Hér færi ég rök fyrir því að bleikjuafbrigðin í Þingvallavatni séu einstaklega vel til þess fallin að rannsaka þróun æxlunareinangrunar í ljósi fyrrgreindra hugmynda. Um þetta er fjallað í fimm greinum í ritgerðinni. Í fyrstu beindist athyglin að tveim afbrigðanna, dvergbleikju og murtu. Niðurstöður staðlaðra eldistilrauna með afkvæmi afbrigðanna og kynblendinga þeirra gáfu til kynna að kynblöndun hefði takmörkuð áhrif á mynstur samdreifni svipfarsþátta er tengjast líkamslögun, tímasetningu þroskunarferla og fæðuatferli (1. grein) og persónuleikaþáttum (2. grein). Á hinn bóginn sýndu athuganir á breytileika í tjáningu gena á fósturskeiði að kynblöndun afbrigðanna gæti haft áhrif á möguleika til þróunar (3. grein). Þá var lagt mat á nokkra þætti sem stuðlað geta að æxlunareinangrun milli afbrigðanna, t.d. þætti sem tengjast vali á hrygningarsvæðum, vali á maka og þroskun kynblendinga (4. grein). Að lokum var samspil vals á hrygningarstað og fósturþroskunar hjá kuðungableikju rannsakað sérstaklega, en hún hrygnir miklu fyrr en hin afbrigðin og er þannig æxlunarlega einangruð frá þeim (5. grein). Athuganir á hrygningarslóð og eldistilraunir með afkvæmi kuðungableikju benda til þess að hún velji að hrygna á stöðum þar sem hitastig er lágt og þroski hægur. Rannsóknir þessar gefa gagnlegt yfirlit um æxlunareinangrun meðal bleikjuafbrigðanna í Þingvallavatni auk þess að vera vegvísir frekari rannsókna á tegundamyndun í ljósi samhengis þroskunar-, vist- og þróunarfræði.

Résumé

La théorie de la divergence par l'évolution de polymorphisme basé sur les ressources qu'exploitent les organismes est une composante importante de la diversification des vertébrés. Cette théorie a récemment été augmentée afin d'inclure les effets d'interactions entre processus écologiques et développementaux (c.-à-d. dans le cadre théorique « eco-evo-devo »). Cependant, cette théorie ne rend pas suffisamment compte de l'évolution de l'isolement reproductif entre populations malgré les récentes avancées faites par les études sur la spéciation. A travers cette thèse, je soutien que les morphes d'Ombles chevalier de Thingvallavatn constituent un système idéal pour étudier comment l'évolution de l'isolement reproductif s'inscrit au sein de la théorie de la divergence par polymorphisme basé sur les ressources. Ma thèse contient cinq études à ce sujet. Premièrement, je présente des travaux sur deux morphes sympatriques qui sont le « petit Omble benthique » (PB) et l'Omble « planctonivore » (PL). Des expériences d'élevage en conditions communes offrent un nombre de preuves limité que l'hybridation influence la structure des covariances entre traits morphologiques ou de comportements alimentaires avec d'autres en lien avec les vitesses de développement (article I) ou entre traits de personnalité (article II). Cependant, des informations sur la variabilité d'expression génétique chez des embryons indiquent que l'hybridation influence la capacité des populations à évoluer par sélection naturelle (article III). Différentes barrières reproductives entre les deux morphes concernant l'utilisation de différents habitats, l'appariement associatif et le développement des hybrides ont aussi été évaluées (article IV). Enfin, l'article V rapporte des études de terrain et des expériences d'élevage explorant les liens entre le choix des habitats pour la reproduction et le développement de la progéniture chez la morphe appelée « grande Omble benthique » (GB) qui diffère des autres morphes par une période de fraie plus précoce. Les résultats suggèrent que les ombles GB choisissent des conditions de températures permettant retarder le développement de leur progéniture. Dans l'ensemble, ces travaux offrent une vue d'ensemble sur l'isolement reproductif entre les morphes d'Ombles chevalier de Thingvallavatn et constituent une première approche pour étudier la spéciation au sein du cadre théorique «eco-evo-devo ».

A mes parents, mes grands-parents, mon frère, mes oncles, mes tantes, mes cousin·e·s et mes petit·e·s cousin·e·s.

Pour les balades dans les bois, les virées à travers champs et les journées à la Marne qui ont façonné mon rapport à la Nature.

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Abbreviations

Age-0: Young of the year juvenile

ddRAD-seq: double digest Restriction site Associated DNA Sequencing

Evo-Devo: Evolutionary Developmental biology

F_1: First generation offspring

F_{ST}: Fixation index, a measure of population differentiation

GLMM: Generalized Linear Mixed-Effects Model

MCMC: Markov chain Monte Carlo

mtDNA: mitochondrial DNA

miRNA: microRNA

mRNA: messenger RNA

LB: Large benthic

PI: Piscivorous

PC: Principal component

PCA: Principal component analysis

PCR: Polymerase Chain Reaction (test)

PL: Planktivorous

PLS: Partial Least Square

SB: Small benthic

SNP: Single-nucleotide polymorphism

RI: Reproductive isolation

Wnt: Wingless/Integrated (signalling pathways)

List of original papers.

This thesis is a collection of three scientific paper. Hereafter, I refer to these papers as follow:

Paper I. Horta-Lacueva, Q. J.-B., Snorrason, S. S., Morrissey, M. B., Leblanc, C. A., & Kapralova, K. H. (2021). Multivariate analysis of morphology, behaviour, growth and developmental timing in hybrids brings new insights into the divergence of sympatric Arctic charr morphs. *BMC Ecology and Evolution*, 21(170), 1–15. https://doi.org/10.1186/s12862-021-01904-8

Paper II. Horta-Lacueva, Q. J.-B., Benhaïm, D., Morrissey, M. B., Snorrason, S. S., & Kapralova, K. H. (2021). Animal personality adds complexity to the processes of divergence between sympatric morphs of Arctic charr. *Animal Behaviour*, 175, 57–73. https://doi.org/10.1016/j.anbehav.2021.02.022

Paper III. Horta-Lacueva, Q. J.-B., Jónsson Z. O., Ponsioen L., Rúnarsdóttir D. Á., Kapralova, K. H. **(**manuscript**)** Dominance determines gene expression variability in embryos of Arctic charr morphs: consequences for hybridization and evolvability.

Paper IV. Horta-Lacueva, Q. J.-B., Snorrason S. S., Lesdalon, R. L. K., Rayssac C. M., Sciannamea M. P., Kapralova, K. H. **(**manuscript**)** Asymmetric reproductive isolation in sympatric Arctic charr morphs revealed by a comprehensive examination of reproductive barriers.

Paper V. Horta-Lacueva, Q. J.-B., Ólafsdóttir, J. H., Finn, F., Fiskoviča, E., Ponsioen, L., Cámara, M., & Kapralova, K. H. (2022). From drones to bones: Assessing the importance of abiotic factors for salmonid spawning behaviour and embryonic development through a multidisciplinary approach. Ecology of Freshwater Fish, (2021), 1–11. https://doi.org/10.1111/eff.12654

During my PhD studies, I also contributed to the following publications, which are not included in thesis:

De la Cámara, M., Ponsioen Lieke, Horta-Lacueva, Q. J.-B., & Kapralova, K. H. (2021). The dynamic ontogenetic patterns of adaptive divergence and sexual and dimorphism in Arctic charr. BioRxiv, 2021.01.15.426104. Retrieved from https://doi.org/10.1101/2021.01.15.426104

Linden, B., Foord, S., Horta-Lacueva, Q. J.-B., & Taylor, P. J. (2020). Bridging the gap: How to design canopy bridges for arboreal guenons to mitigate road collisions. *Biological Conservation*, *246*(March). https://doi.org/10.1016/j.biocon.2020.10856

Author's contributions.

Paper I. QJH conceived the study, reared the specimens, collected the data, conducted the analyses and drafted the manuscript. SSS coordinated the field work, produced the embryos and critically revised the manuscript. MBM provided guidance for the data analyses, contributed to the biological interpretations of the results and reviewed the manuscript. CAL developed the rearing setup, contributed to designing the behavioural experiments and reviewed the manuscript. KHK established the crossing design, produced the embryos, organized the logistics of the transfer and the maintenance of the specimens, provided guidance during the experiments and critically revised the manuscript.

Paper II. QJH, KHK, SSS, DB conceived the experiments. DB developed the behavioural tests and realised the testing setup. QJH collected the data. SSS, KHK and QJH collected the parent specimens and conducted the gamete crossing. QJH and KHK reared the offspring. QJH conducted the data analyses under the guidance of MBM, and drafted the manuscript. QJH. MBM DB, KHK, SSS critically revised the manuscript.

Paper III. KHK and ZOJ conceived the experiments. KHK conducted the molecular work. KHK, ZOJ, LP, DAR and QJH collected the wild specimens and crossed the gametes. KHK and QJH reared the embryos. LP stained the embryos and collected the phenotypic data. QJH conducted the data analyses. DAR contributed to data preprocessing and to the differential expression analyses. QJH drafted the manuscript. KHK, ZOJ, DAR and LP critically revised the manuscript.

Paper IV. SSS, KHK and QJH conducted the fishing surveys and the gamete crossing. KHK and QJH reared the embryos. QJH and KHK designed the mate choice experiment. QJH conducted the mate choice experiment. RLKL collected and preprocessed the mate choice experiment data, and conducted exploratory analyses. CMR, MPS and RLKL collected the morphological data. CMR digitized and preprocessed the morphological data, and conducted exploratory analyses. QJH analysed the data and wrote the manuscript. KHK and SSS revised the manuscript.

Paper V. JO, KK and QH conceived and designed the study. JO, QH and LP performed the field work. KK conducted the laboratory work. EF and FF collected and preprocessed the data from the video records. QH and MC analyzed the data. Wrote the paper: KK, QH, MC, LP.

Acknowledgements

I first would like to thank the taxpayer for providing the financial resources for the projects covered in this thesis and for my salary.

I am very grateful to my supervisors Kalina Kapralova and Sigurður Snorrason for supporting me with administrative, conceptual and technical help through my PhD studies, and especially for being amazing mentors. Many thanks to Skúli Skúlason, Neil Metcalfe and Michael Morrissey for their supervision, for the time they invested in providing theoretical guidance, and for welcoming me in their respective institutions.

Special thanks to my close collaborators Marina de la Cámara, Lieke Ponsioen, and Fia Finn. A big thank you to Arnar Pálson, Dagný Rúnnarsdóttir, Marcos Lagunas, Zophonías Jónsson, Sigríður Rut Franzdótir, Sébastien Matlosz (also adventure buddy), Han Xiao, Sarah Steele, Nahal Eskafi, Gregoire Gaillet, Thierry Chanet, Sylvain Moinard, Mieke Luijten, Jóhannes Guðbrandsson, Gréttar Guðmundsson, Célia Pradon, Laura-Marie von Elm, Hrefna Sigurjónsdóttir, Kristján Þórhallsson, Guðbjörg Jónsdóttir, Oren Raz, Léa Jerman-Plesec, Styrkár Þóroddsson and Edite Fiskoviča for all their help, and even for their implicit support and indirect input during my PhD studies. Also, thanks to Eduardo Rodriguez for his friendliness and help during lab work. I am grateful to Ian Dworkin for taking the time advise me during my PhD projects. Many people I met in conferences and during workshops have also contributed to my thinking.

Thank you Jónína Ólafsdóttir for your collaboration in setting up and supervising an awesome field study. Many thanks to Anna María Einarsdóttir for making me a diver and providing me with diving material for fieldwork.

When collecting and analysing data during my PhD, I had the privilege of being helped by Chloé Chavoix, Rebecca Lesdalon, Cécile Rayssac, Marie Sciannamea and Alias Desclos. I also enjoyed setting-up a bachelor project with Helena Gylfadóttir.

I am also very thankful to Elizabeth A. Mittell for taking me under her wing when visiting St Andrews, and to all the scientists, students and staff members who welcomed me there. Furthermore, I was very pleased by the enthusiastic exchanges I had with scientists and students during my short visit at the University of Glasgow.

Thanks to Hlynur Sigurgíslason, Snorri Davíðsson, Sigrún Reynisdóttir, Karl Arnason and Þuríður Sigurjónsdóttir for helping me navigating into the administrative and technical mess of academia. Many thanks to Þórhallur Aðalsteinsson for making sure that I benefited from the best working conditions ever (unlimited fresh coffee, friendliness and permanent assistance to people locked outside of the office or forgetting dirty lunchboxes around).

My stay at Hólar was a great professional and human experience thanks to Bjarni K. Kristjánsson, David Benhaïm (both as a collaborator and a swimming partner), Camille Leblanc, Kári Árnason, Rakel Þorbjörnsdóttir, Christian Beuvard, Mori Morks, Tina dirr, Ilona Laine, Jesse De Vries, Wolf van Lier, Marlène Kundruhn, Michael and Krystal Galloway, Christina Anaya, Doriane Combot, Stefán Óli Steingrímsson, Sven Winston, Amber Christina, Herdís Ólína Hjörvarsdóttir, Lydia Tobias, Anett Reilent, Samantha V.

Beck and many others. I also had a great pleasure working with Joris Philip and Marion Dellinger and interact with Kasha Strickland and Alessandra Schnider. I am also grateful to Janette Boughman for her advice for my projects.

I also received much emotional support from my fellow PhD students, with who I shared great moments at the office and outside the university, Agnes Kreiling, Hildur Magnúsdóttir, Ragnhildur Guðmundsdóttir, Gotje von Leesen, Maite Cerezo, Charles C. Hansen, Anna Selbmann, Will Butler, Ian Klupar, Ayça Eleman and Axel W. Einarsson. Also many thanks to Denis Warshan, Jake D. Goodall, Noémie Boulanger-Lapointe, Alejandro Salazar and Hannah Reynolds for postdoc'ing around.

I am very grateful Sindri Gíslason, Hanna María Kristjánsdóttir, Halldór P. Halldórsson, Hermann D. Guls, Sölvi R. Vignisson and Valdís Sigurðardóttir for welcoming me at Sudurnes Science and Learning Center and for helping me with my stay and for building the experimental setup. I also received very helpful advice from Daniel Coaten when setting up this experiment.

I am very thankful to Finnur Ingimarsson and Haraldur R. Ingvason (Natural History Museum of Kópavogur) for their help during adverse fieldwork conditions. I also thank Jóhann Jónsson, Rósa Jónsdóttir at their dog Kátur for their very warm welcome on their farm during fieldwork.

Je ne remercierais jamais assez mes parents pour leur soutien financier et moral tout au long de mes études. Je suis aussi très reconnaissant envers mes collègues et amis, volontaires et salariés de la Ligue de Protection des Oiseaux Champagne-Ardenne, qui m'ont formé en tant que naturaliste, m'ont beaucoup appris sur le plan personnel et m'ont aidé à partir faire un doctorat. Merci à mes professeurs·es, mes maitres de stage et mes instituteurs·ices qui depuis la maternelle ont été m'ont inspiré et motivé à étudier les sciences.

I am thanking my Bachelor and Master supervisors Ian Ghaigher and Birthe (Bibi) Linden for encouraging me in doing sciences, for being a great source of inspiration, for giving me the opportunity to work on great projects and for supporting me during the following steps of my education. I am also grateful to Kyle Stuart, Jabu Linden, Vümboni Harry Msimango, Lorène Garnier, all the staff of Lajuma Research centre and the students and friends I met there during these parts of my studies. The help in I received from them, and the personal experience shared with them keep influencing me.

I thank my beloved Ingeborg Klarenberg for her encouragement and for grabbing me away from my studies for *gezellig* moments and awesome adventures – together with her partners in crime Ed, Basha, Maartje, Hrafn, Jóhanna and Gunnar.

I also thank my flatmates for their friendliness and support all along my PhD studies.

Finally, I thank the person I forgot mentioning and in front of who I may feel awkward because of that.

Thank you all for having made these PhD studies a great adventure.

1 Introduction.

1.1 Time to bring speciation to Eco-Evo-Devo.

Over the past decade, voices have raised to either announce or disclaim a paradigm shift on the ways to view and study evolution through natural selection (Laland et al. 2014; Skúlason et al. 2019; Svensson 2021). To say the least, many for the last conceptual and empirical advances in evolutionary biology go beyond sheer descriptions and predictions of allele frequency changes under evolutionary forces. Much of this work relates to the evolutionary developmental biology tradition (Evo-Devo), and touches upon the importance of development in modulating phenotypic variation, like the role of phenotypic plasticity in shaping evolutionary paths (Hallgrimsson et al., 2019; Pfennig et al., 2010; Uller, Moczek, Watson, Brakefield, & Laland, 2018). Major progress has also been made toward the appreciation of how adaptive phenotypic changes feed back to ecology (Hendry, 2016; Sih, Cote, Evans, Fogarty, & Pruitt, 2012). Given this state of knowledge, several authors proposed a comprehensive framework on the interactions between ecology, developmental biology, and adaptive evolution (Eco-Evo-Devo; Gilbert, Bosch, and Ledón-Rettig 2015), which is receiving increasing attention (Figure 1). More recently, an attempt was made to improve the explanatory power of the Eco-Evo-Devo framework through the resource polymorphism theory (Skúlason et al. 2019).

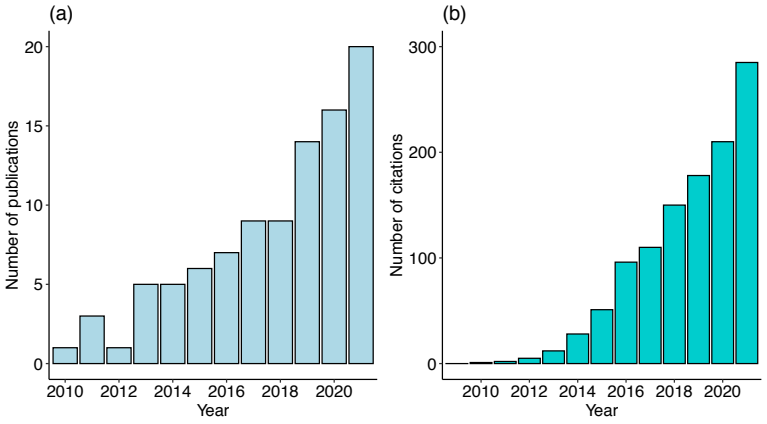


Figure 1. *Use of Eco-Evo-Devo framework over time. Bar plot of (a) the number of publications and (b) the number of citations per year of research papers containing the keywork "eco-evo-devo". Web of Science search over the 1970-2021 period. Note the different scales between the two panels.*

The theory of resource polymorphism was originally proposed to explain the evolution of adaptive divergence among many vertebrates (Skúlason and Smith 1995). It is generally

presented as a continuous process, starting with the reduction of intraspecific competition as populations exploit new trophic resources, followed by the enhancement of phenotypic diversity in response to divergent selection, and resulting in an increasingly stable polymorphism as gene flow reduces (Figure 2; Smith and Skúlason 1996). Integrating this model into the Eco-Evo-Devo framework better entails the evolution of phenotypic plasticity or the changes in selection regimes during divergence (Skúlason et al. 2019). However, one weakness to this verbal model is that, while the reduction of gene flow along divergence is acknowledged as a key aspect of this extended theory of resource polymorphism, there is no explicit mechanistic explanation on the evolution of reproductive isolation throughout the divergence processes, nor of its interactions with developmental and ecological processes.

Integrating reproductive isolation into this extended theory of divergence through resource polymorphism is timely. Indeed, breakthroughs in understanding speciation processes were accomplished while the conceptual bases of Eco-Evo-Devo were being laid. The modern thought on speciation have recently been marked by the age of "omics" (Foote, 2017; Pavey, Collin, Nosil, & Rogers, 2010; Seehausen et al., 2014; Tigano & Friesen, 2016) and by a (re)focus on the role of divergent selection in the evolution of reproductive isolation (Sobel et al. 2010; Nosil 2012a; Schluter 2009). Perhaps a major conceptual step was made by the apprehension of the speciation continuum (Kulmuni, Butlin, Lucek, Savolainen, & Westram, 2020; Nosil, 2012b; Stankowski & Ravinet, 2021). The speciation continuum is analogous to the model of divergence via resource polymorphism in a way that both refer to continuous processes. However, the speciation continuum is acknowledged for not being unidirectional nor unidimensional, and for involving various evolutionary paths that include the interactions of different reproductive barriers (Kulmuni et al., 2020; Stankowski & Ravinet, 2021). Further contrasts with Skúlason and colleagues' model of divergence by resource polymorphism emerge when considering that the progression of genetic divergence is not linear along the speciation continuum and is poorly explained by phenotypic levels of differentiation and ecological parameters (Matute & Cooper, 2021; Rabosky & Matute, 2013; Stankowski & Ravinet, 2021). Genetic divergence also do not evolve at the same rate among taxa and varies depending on the type of barriers involved (Matute & Cooper, 2021; Rabosky & Matute, 2013; Stankowski & Ravinet, 2021). Furthermore, the feedbacks between the mechanisms of reproductive isolation, development and ecology remain under-evaluated (Figure 2).

This explanatory gap is especially noticeable regarding the effects of hybridization. Many mechanisms are at play during hybridization – like the breakdown of coadapted alleles (Dobzhansky, 1936), heterosis (Ackermann, Rogers, & Cheverud, 2006), transfers of standing genetic variation (Seehausen et al., 2014) and genetic architecture-dependent allelic changes (Arnegard et al., 2014) – and these mechanisms may have various evolutionary consequences, ranging from hybrid incompatibilities that facilitate phenotypic divergence between introgressed populations, to the colonisation of new niches (Abbott et al., 2013; Seehausen, 2004). Moreover, little is known about how these effects of hybridisation translate into developmental processes. While developmental deficiencies stemming from genetic incompatibilities have become a standard in speciation research (Mack, Campbell, & Nachman, 2016; Rice & McQuillan, 2018; Thompson et al., 2022), attempts to establish how hybridization interferes with classical Evo-Devo processes modulating phenotypic variation like developmental stability, canalisation, or genetic assimilation, are very scarce.

Fishes from postglacial freshwater systems are promising models to study Eco-Evo-Devo dynamics with regards to reproductive isolation. Across the Northern hemisphere, many freshwater fish populations exhibit a wide range of phenotypic variations within the same geographic areas (Smith and Skúlason 1996; Skúlason et al. 2019; Hendry 2009; Lackey and Boughman 2017; Doenz et al. 2018; Schluter 1996; Ólafsdóttir, Ritchie, and Snorrason 2006). These fish are illustrative examples of resource polymorphism, often corresponding to morphs segregating between benthic and pelagic habitats following the colonisation of deglaciated lakes. These morphs often differ in diet, morphology, life-history traits and behaviour (Smith and Skúlason 1996; Skúlason et al. 2019). Typical examples include benthic and limnetic sticklebacks, *Gasterosteus sp*. in Lakes Paxton and Enos, Canada (Lackey & Boughman, 2017), littoral and pelagic Eurasian perches, *Perca fluviatilis* in Lake Erken, Sweden (Marklund et al., 2019) or the six incipient species of Alpine whitefish, *Coregonus sp*. occupying various water depths in Lakes Thun and Brienz, Switzerland (Doenz et al., 2018). Because of recent evolutionary histories, a wide range of ecological conditions and geographic setups, and varying magnitudes of phenotypic differences and phenotypic plasticity, postglacial fishes were presented as ideal models for the extended theory of resource polymorphism (Skúlason et al., 2019). Strikingly, these systems occupy positions all along the speciation continuum, presenting single populations with continuous variation, discrete varieties with incomplete reproductive isolation as well as different species (Doenz et al., 2018; Hendry, 2009; Lackey & Boughman, 2017), which also provides remarkable opportunities to study the evolution of reproductive isolation.

One of the most striking cases of resource polymorphism is found in lake Thingvallavatn in Iceland. Four Arctic charr morphs with contrasting ecology and extensive phenotypic differences coexist in this postglacial lake (Figure 3). Two morphs correspond to a benthic and an epibenthic ecotype: the small- (SB) and the large benthic charr (LB), respectively. The two other morphs are limnetic ecotypes: the planktivorous (PL) and the piscivorous charr (PI, Snorrason et al. 1994, 1989). All four morphs differ in habitat use, diet, head and body morphology, life history and parasitism (Sandlund et al., 1992). Three morphs (the LB-, PL- and SB-charr) have extensive genetic differences across their genomes, while the PI-charr is suspected to originate from ontogenetic niche shifts in PL-and LB-charr (Brachmann, Parsons, Skúlason, & Ferguson, 2021; De la Cámara, unpublished; Jóhannes Guðbrandsson et al., 2019; Kapralova et al., 2011; Kapralova et al., 2013; Malmquist, Snorrason, & Skúlason, 1985). The LB-charr is reproductively isolated from the other morphs by its unusually early spawning time, while there is no obvious spatiotemporal barrier to gene flow between PL- and SB-charr (Skúlason, Snorrason, Noakes, Ferguson, & Malmquist, 1989). Hence, the Arctic charr of Thingvallavatn is a prime model to study speciation within an eco-evo-devo framework by presenting extensive phenotypic variations involving contrasting developmental trajectories, a recent evolutionary history, and most likely different reproductive barriers.

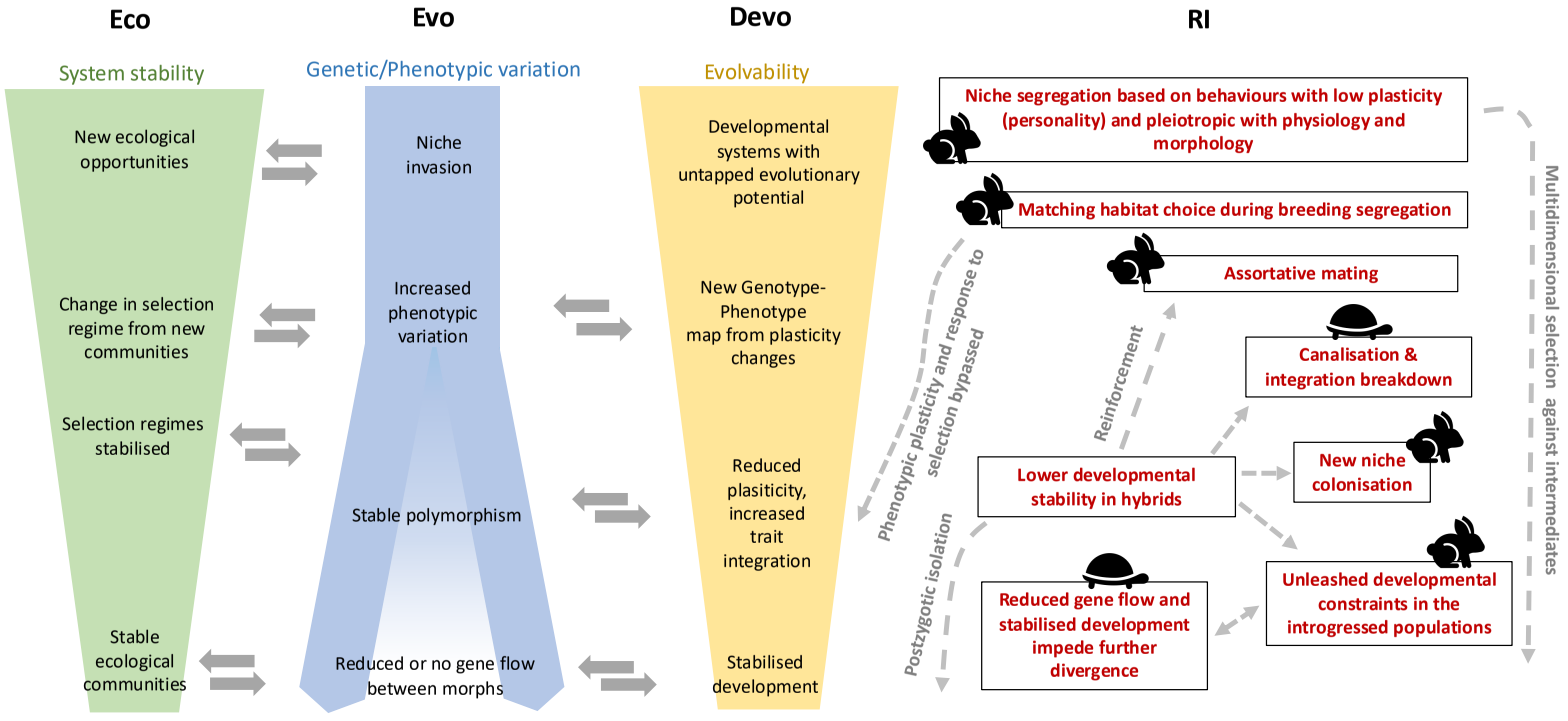


Figure 2. *A schematic illustration of how mechanisms related to reproductive isolation may disrupt the extended model of resource polymorphism (non-exhaustive). Left side: simplified illustration of the verbal model from Skúlason and colleagues (2019). Right side: mechanisms and processes linked to reproductive isolation (RI). Hares: mechanisms or processes facilitating or by-passing Eco, Evo or Devo processes during divergence. Tortoises: mechanisms or processes impeding or breaking down divergence.*

After giving a brief overview of the knowledge acquired by four decades of studies on the Arctic charr of Thingvallavatn, I will discuss how this system presents unique opportunities to study Eco-Evo-Devo processes in relation to reproductive isolation. I will focus on three ways reproductive isolation may interfere with the extended verbal model of resource polymorphism. Firstly, I will present how phenotypic divergence and reproductive isolation may rapidly arise together through behaviour. Secondly, I will discuss how hybridization may affect the changes in evolvability during divergence. Finally, I will introduce matching habitat choice and its potential to induce reproductive isolation while by-passing the evolution of phenotypic plasticity.

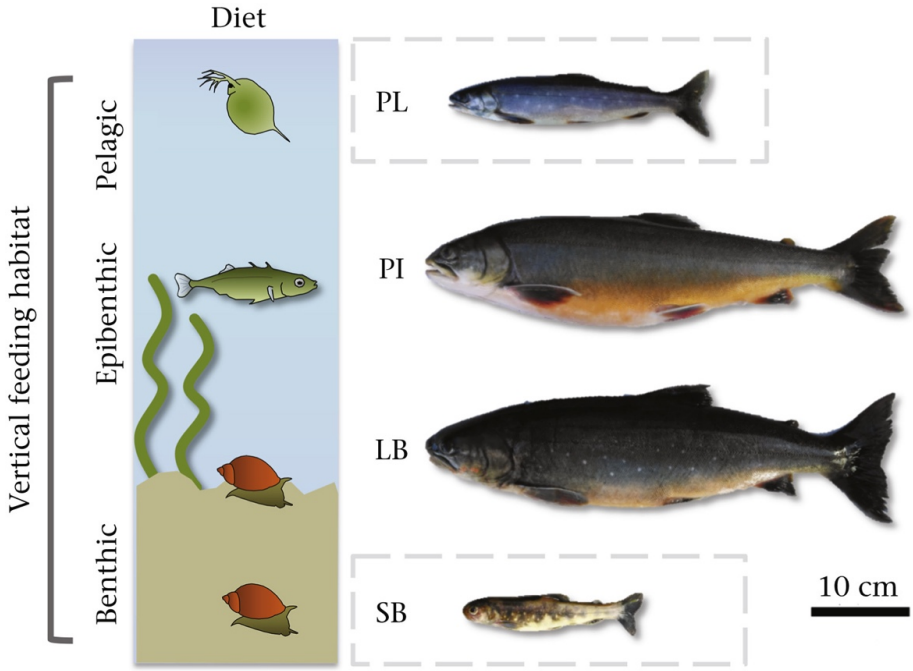


Figure 3. *The four morphs of Thingvallavatn with their foraging habitat and main prey depicted on the left (from top to bottom: planktonic crustaceans, fish, freshwater snail). All four specimens were captured together at the same location. PL: planktivorous; PI: piscivorous; LB: large benthic; SB: small benthic charr. The two morphs with presumed spatiotemporal overlap during spawning (PL and SB) are highlighted with dashed lines. From Horta-Lacueva et al. (2021).*

1.2 Eco-Evo-Devo of the Arctic charr in Thingvallavatn.

1.2.1 Ecological set-up of Thingvallavatn: the "Eco".

Thingvallavatn is an oligotrophic lake sitting in a graben of the Mid-Atlantic ridge in SW Iceland (area = 83km^2). The "modern" lake formed following the last glacial retreat about 11,000 years ago (Pétursson, Norðdahl, & Ingólfsson, 2015; Sæmundsson, 1992). Soon after this, several geological events drastically reshaped the lake, the most prominent being the formation of the Skjadbreidur- and Eldborgir lavas (Sæmundsson, 1992). The Eldborgir lava covers a large parts of the present lake bottom and characterizes the structural properties of the littoral zone along the northwestern, northern and eastern shores (Sæmundsson, 1992). These lava fields provided a barrier to surface inflow from the north and the east, resulting in the lake being mostly fed by percolating groundwater from the Thórisjökull and Langjökull glaciers (Adalsteinsson, Jónasson, & Rist, 1992; Jónasson, 1992). The Eldborgir lava also dammed the outlet river Efra-Sog around 10,200 years ago (9,130 ± 260 ^{14}C y BP; Kjartansson, 1964). The lava dam elevated the water level by about 25 m, but as it eroded approximately between 7,300 and 6,000 years ago, the water level dropped by some 11m (Sæmundsson, 1992). The last eruption event in the lake dates from about 1,900 year ago when the Nesjavellir lava was formed and a tuff cone rose from the depth of 100m forming the island Sandey in the middle of the lake.

Thingvallavatn has been subject to major anthropogenic disturbances during the last century. The most significant of these events is the construction in 1959 of a dam diverting water from the outlet river Efra-Sog for the Steingrímsstöð power plant. This construction destroyed the extremely productive ecosystem of Efra-Sog, including the habitats of the American black-fly (*Simulium vittatum*), which was an important early summer food resource for brown trout juveniles and Arctic charr in the southern part of the lake. The damming of the river also destroyed spawning grounds of brown trout (*Salmo trutta*) at the outlet and contributed to the collapse of this population in the lake (Jónasson, 1992).

The lake is characterized by a wide pelagic zone (volume = 2,855 Gl; mean depth = 34m; max. depth = 114m). The benthic structure of Thinvallavatn is complex, which has probability facilitated the divergence of Arctic charr by providing multiple ecological opportunities. The benthic zone is characterized by a high microhabitat complexity, mainly because of the structure of uneroded lava in the surf zone (0-2.5 m deep ; ~3.8km^2) and in a "stony littoral" zone (2.5-10m deep ; ~11.4 km^2; Malmquist, Antonsson, Gudbergssion, Skúlason, & Snorrason, 2000). Note that these microhabitats are associated with a high diversity and abundance of macroinvertebrates (Malmquist, Antonsson, Gudbergssion, Skúlason, & Snorrason, 2000). The benthic habitats of the lake also include a sublittoral zone with diatomic gyttja (~10-20m deep ; 15km^2) variably covered by stands of the green alga *Nitella opaca* that can reach heights of 70-80cm in the autumn, and a profundal zone covered by a diatomic gyttja substrate (~25 m and deeper ; area bellow 30m = 70km^2; Sandlund et al. 1992; Adalsteinsson, Jónasson, and Rist 1992).

Only three fish species occupy the lake: the Arctic charr, the threespine stickleback (*Gasterosteus aculeatus*) and the brown trout (*Salmo trutta*). Threespine stickleback are mostly found in the *Nitella* zone where they can reach extremely high densities (Kairesalo et al. 1992). Sticklebacks also live in the stony littoral and the surf zone where they are in very low densities compared to charr of comparable size (Sandlund et al. 1988, Ólafsdóttir et al 2007). Two stickleback morphs have been described in Thingvallavatn, and are distributed between the stony littoral zone and the *Nitella* habitats (Kristjánsson, Skúlason, & Noakes, 2002; Ólafsdóttir et al., 2006).

The brown trout, a top predator, collapsed during the second half of the twentieth century. This collapse is most likely resulting from the destruction of the spawning grounds in the outlet river during the construction of the power plant (Adalsteinsson et al., 1992; Jónasson, 1992). However, another major spawning population is found in the Öraxá river (northen part of Thingvallavatn) and what caused the collapse of brown trout in the whole lake remains unknown (Lagunas *et al*., 2022 ; Snorasson, personal communication). While the trout populations remained modest for decades, a 2019 survey reported an impressive rebound that may even overtake the Arctic charr populations (Snorrason, unpublished). Other predators of Arctic charr may also include piscivorous waterbirds, mostly being represented by the red-breasted merganser (*Mergus serrator*) and the great northern diver (*Gavia immer*, Magnússon 1992). PL-charr were also subjected to intensive commercial exploitation from the 1930's to the 1980's (Snorrason et al. 1992).

The four charr morphs show contrasting difference in feeding habitats (Figure 2). The SB-charr is restricted to the stony littoral zone and lives within the interstitial spaces of the lava (Sandlund et al., 1987). The LB-charr is epibenthic and occupies the stony littoral and the *Nitella* zones. PI-charr mostly forage above the stony littoral and the *Nitella* zones, but are also found in low numbers in pelagic habitats. The PL-charr have the most widespread distribution within the lake, but exhibit dynamic patterns of habitat use (Sandlund et al., 1987). The PL-charr dominate the pelagic and epibenthic zones with the exception of the shallow littoral areas. They are present at all depths, but changes in their vertical distribution occur through the year and between habitats (Sandlund et al., 1992, 1987). In June, PL-charr were observed to be mostly located at a depth of 20-26m in the pelagic zone, but were most abound near the bottom of the *Nittella* zone (Sandlund et al., 1987). However, high densities of PL-charr were reported near the surface of the pelagic zone in August. This density decreased gradually with depth but increased again near the bottom (Sandlund et al., 1992, 1987). There is also a partial habitat segregation between age classes (and size) in PL-charr, with charr in age group 1-3 being mostly found near the bottom. Sandlund and colleagues (1987) also reported differences in habitat use between the sexes, with females being more abundant than males in the pelagic zone.

The young of the year Arctic charr (age-0 charr) live in the surf zone – and most likely also in the stony littoral zone – from May to November, where they feed mostly on chironomids (Sandlund et al., 1988). Drop in fish densities in the surf zone that coinciding with catches of age-0 charr on the pelagic zone at 10-20m depth suggests that the young of the year PL-charr migrate towards the pelagic and the epibenthic habitats in late summer (Sandlund et al., 1988).

Diet is another major component of polymorphisms in the Arctic charr of Thingvallavatn (Figure 2; more details in Malmquist *et al*. 1992). Adult LB- and SB-charr feed extensively on the freshwater snail *Radix peregra*, although insects larvae and pupae can constitute most of the LB-charr diet in the *Nitella* zone in summer, and in the littoral zone in late spring (Malmquist et al. 1992; Sandlund et al. 1992). *Radix peregra* dominate the secondary production of the littoral zone, with a stable biomass through the year, but a peak in the distribution of large adults snails in May-June seems to be utilized by both LB- and SB-charr (Snorrason, 2000). The PL-charr prey mainly on *Daphniae* and *Cyclopidae*, two planktonic crustaceans with high variations in availability as a result of diel migration cycles and seasonal changes of instar abundances (Antonsson, 1992). Finally, the PI-charr hunt stickleback in the stony littoral and the *Nittella* zones, but may be found eating juvenile Arctic charr in the deep pelagic zone in late summer (Sandlund et al., 1992).

1.2.2 Evolutionary history and selection regimes: The "Evo."

The four morphs of Arctic charr in Thingvallavatn have evolved complex phenotypic differences (Sandlund et al., 1992). The morphological characteristics of the LB-and the SB-charr include a round snout, subterminal mouth, a stocky body and long pectoral fins (Snorrason et al., 1994). These two morphs differ in size and colour patterns (Snorrason et al., 1994). The PL- and the PI-charr are characterised by a pointed snout, a terminal mouth, a fusiform body, and differ in size, jaw robustness and teeth size (Snorrason et al., 1994). The morphological differences are concomitant with behavioural variations as PL-charr spend more time hovering than SB-charr, and exhibit higher feeding activity and capture rate when offered planktonic food (Malmquist, 1992; Skúlason, Snorrason, Ota, & Noakes, 1993). Common-garden experiments showed genetic bases for behavioural differences between PL- and SB-charr (Skúlason et al., 1993). They also revealed complex foraging strategies in PL-charr in relation to prey density, which might be resulting from trade-offs involving energetic costs and/or predation risk (Skúlason et al., 1993). The selection regimes behind these phenotypic differences haven't been deeply investigated. However, Franklin and colleagues (2018) studied natural selection for size and body shape between PL- and SB-charr. They reported lower growth in wild PL- and SB-charr with intermediate values for mouth position and body shape, and provided from mortality estimates evidence for divergent selection on body size between the two morphs. Interestingly, path analyses from Franklin and colleagues (2018) indicated that body shape influenced growth through diet in the PL-charr only, mostly via the indirect effect of parasitism, so trophic and non-trophic sources of divergent selection might be at play in this system.

Profound changes in understanding the population structure and the evolutionary history of the Arctic charr of Thingvallatn have occurred as molecular and bioinformatic techniques developed. A first study on allozyme frequencies (Magnusson & Ferguson, 1987) have established that all four morphs are closely related (Nei's $D \leq 0.001$), and that SB-charr significantly differ from the three other morphs (in heterogeneity of number of alleles at one out of five loci, *Est2*). However, a subsequent study on restriction fragment length polymorphism in mtDNA (Danzmann, Ferguson, Skúlason, Snorrason, & Noakes, 1991) reported a high degree of genetic similarity among morphs, suggesting that they do not constitute distinct lineages. Slightly later, higher resolution tests for genetic polymorphisms (mtDNA restriction sites and minisatellites) suggested some genetic heterogeneity between

ecotypes pairs (SB- with LB-charr and PL- with PI-charr) but not among morph within ecotypes. A major turn in considering the population structure of these morphs was made by a ten microsatellite loci study reporting modest genetic differentiation between SB- and PL-charr (overall F_{ST} = 0.04 ; 95% CI = 0.01 – 0.06 ; Kapralova et al. 2011). Shortly after, strong differentiation at two immunological candidate genes (*Cath2* and *MHCII*α) was observed among the PL, SB- and LB-charr (Kapralova et al. 2013). Lately, a transcriptomic study on 22 candidate variants (single nucleotide polymorphism) spread over the genome provided further support to the strong genetic differentiation between the PL-, LB- and SB-charr, while many PI-charr were estimated as genetically similar to either the PL- or the LB-charr (Guðbrandsson et al., 2019) . Most recently, SNP array and ddRAD-seq genotyping revealed strong genetic structuring among PL-, SB- and LB-charr (Brachmann et al. 2021; De la Cámara, *in prep*.).

The recent evolutionary history of the Arctic charr in Thingvallavatn has been pinpointed by microsatellite-based population genetic studies showing that the Arctic charr in Iceland originate from the postglacial colonisation of a single lineage, the Atlantic lineage (Wilson et al. 2004). The sympatric origin of the four morph of Arctic charr in Thingvallavatn has been suggested in an early genetic study (mtDNA fragment length polymorphisms) investigating population differentiation between lakes vs. within lake in Iceland (Volpe & Ferguson, 1996). However, the most parsimonious evolutionary scenario according to coalescent simulations with the microsatellite data from Kapralova and colleagues (2011) involved short periods of geographic isolation between the PL- and the SB-charr (micro-allopatric scenario, Kapralova et al., 2011).

The evolution of reproductive isolation in the Arctic charr of Thingvallavatn have not been thoroughly investigated so far. However, some insight can be gained from next-generation sequencing data. First, the F_{ST} values of variants plotted on genomic coordinates show widespread and very heterogeneous levels of genetic divergence across the genome (Guðbrandsson et al., 2019), a pattern that is characteristic of sympatric species at a late point along divergence (Seehausen et al., 2014). Second, results from SNP array genotyping analyses suggest 3.2% of hybrids among the PL- and LB-charr, and 9.5% among the PL- and SB-charr, suggesting moderate gene flow amongst the three morphs (Brachmann et al., 2021). Little is known about the reproductive barriers at play, but the early spawning season of LB-charr is indicative of temporal isolation from both the PL- and the SB-charr (Skúlason et al. 1989). Mature first-generation (F_1) hybrids can easily be reared in laboratory, suggesting modest or absent intrinsic post-zygotic barriers (De la Cámara, Ponsioen, Horta-Lacueva, & Kapralova, 2021). However, common-garden experiments reported some indications for extrinsic postzygotic insolation between PL- and SB-charr as biased body growth towards the maternal morph and intermediate number of fin rays in F_1 hybrids (Eiríksson, Skúlason, & Snorrason, 1999). Such studies also showed that cranial shapes in F_1 hybrid juveniles appeared to be similar to the maternal morphs (Skúlason et al. 1996), although many embryos exhibited extreme ventral head shapes (Kapralova 2014). Clearly, much work is needed to establish the extent of reproductive isolation among the charr morph as well as the nature and the relative strength of the underlying reproductive barriers.

1.2.3 Developmental aspects of divergence in the Arctic charr of Thingvallavatn: the "Devo".

The first common-garden experiments on the Arctic charr of Thingvallavatn have set the bases for clarifying the role of development in phenotypic diversification (Eiríksson et al., 1999; Skúlason, Snorrason, et al., 1989). These studies demonstrated that genetically based differences in head shape among the four morphs were apparent from the onset of exogeneous feeding (Skúlason, Noakes, & Snorrason, 1989). Early common-garden experiments also showed genetically based variations in growth rate between the four morphs, the LB-charr growing the fastest and the SB-charr growing the slowest (Skúlason et al. 1996; Eiríksson, Skúlason, and Snorrason 1999). The variations in growth rate were apparent before the onset of exogeneous feeding, emphasising the role of genetic and early maternal effects (Skúlason et al. 1996; Eiríksson, Skúlason, and Snorrason 1999).

Heterochrony in bone development was mentioned from early on as a potential mechanism at the origin of the cranial variations between the four morphs (Skúlason, Noakes, et al., 1989). This claimed was mostly based on observations that the adult SB-charr morphology corresponded to embryonic or juvenile characters in salmonids, suggesting that the retention of early traits (*i.e.*, paedomorphosis) may be involved in the phenotypic differentiation of the charr morphs (Skúlason, Noakes, et al., 1989). Paedomorphosis is a remarkable example of the interactions between developmental and evolutionary processes, which have been proposed as a catalyst of adaptive divergence in other taxa (Bhullar et al., 2012), making heterochrony in development a very tempting hypothesis to explain the developmental origin phenotypic diversity in the Arctic charr of Thingvallavatn. However, more recent common-garden experiments showed on that embryos of all four morphs have rather similar ontogenetic trajectories of their craniofacial morphology, although LB-embryos significantly differed from PL- and SB-charr in several aspects of such trajectories (Kapralova et al., 2015; Ponsioen, 2020). In free swimming embryos, cranial bones seem to ossify at the same developmental time points in PL- and SB-charr, when some of these bones already differ in shape (Ponsioen, 2020).

A more complete picture of heterochrony in development can now be obtained from gene expression studies in early embryos, perhaps the most investigated aspect of evolution in the Arctic charr of Thingvallavatn in the recent years (Kapralova et al. 2014; Ahi et al. 2013; Guðbrandsson et al. 2016; Ahi et al. 2014; Guðbrandsson et al. 2018). RNA-seq analyses on LB-, PL- and SB-embryos – during a developmental period spanning most of cartilage formation of cranial bones – revealed more than a thousand transcripts with different expression between morphs (Guðbrandsson et al., 2018). Moreover, about eight thousand transcripts differed in expression across time and among morphs (Guðbrandsson et al., 2018). Differential expression in several genes with putative roles in craniofacial morphogenesis (e.g., *mmp2*, *sparc*, *eif4ebp1*) was validated by quantitative real-time PCR (Guðbrandsson et al. 2018; Ahi et al. 2013). Expression in these genes mostly differed between the benthic morphs (SB- and LB-) and the limnetic PL-morph to varying extents along embryo development (spanning the period of cartilage formation). Further studies have established that *mmp2* and *sparc* are part of a larger gene co-expression module, which includes genes involved in morphogenesis and highly expressed in the cartilaginous precursor of the lower jaw and the pharyngeal arches (Ahi et al., 2014). More work also identified the aryl hydrocarbon receptor pathway as an important modulator of the early

ontogeny of cranial differences between the benthic (SB- and LB-charr) and the limnetic morphs (PL- and PI-charr; Ahi et al. 2015). Besides these discoveries on coding RNAs, Kapralova and colleagues (2014) identified differential expression in several microRNAs between embryos of SB-charr and a domesticated charr strained, and at different developmental time point during the formation of cranial bones cartilage. Such findings indicate that small noncoding RNAs may contribute to the development of phenotypic variations in the Arctic charr of Thingvallavatn. Altogether, the information brought by gene expression studies show that the ontogenetic differences among the Arctic charr of Thingvallavatn operate as early as during embryo development, and are related to the regulation of multiple pathways, at least during early development.

While gene expression studies provided accumulating evidence for morphological differentiation in embryos, the evolution of phenotypic plasticity later in life has also been investigated. In a landmark common-garden experiment, Parsons and colleagues (2010) reported higher changes in shape variation over ontogeny within LB-juveniles than within PL-and SB-juveniles. Subjecting the charr juveniles to either limnetic or benthic- mimicking diets also showed that the reduction of shape variation over ontogeny was facilitated by « native » food. These results indicate that the three morphs have evolved differences in the regulation of phenotypic variability through development (*i.e.,* canalisation ; Parsons et al., 2010). A companion study also demonstrated that the benthic vs. limnetic-like diets accounted for a lesser proportion of shape changes in the PL- and LB-charr than in two other morphs from the Icelandic Lake Vatnshlidarvatn (the “brown” and the “silver” morphs), suggesting that the PL- and LB-charr of Thingvallavatn have undergone stronger canalisation (Parsons, Sheets, Skúlason, & Ferguson, 2011). In a follow-up study on the same material, Küttner and colleagues (2014) showed that the magnitude of the phenotypic response to diet treatment in LB-and PL-charr (and in the two Lake Vatnshlidarvatn charr morphs) was altered by their genetic architecture: diet-related shape changes in cranial traits were linked to several quantitative trait loci (QTL) from different linkage groups among morphs.

Maternal effects are also important aspects of the development of phenotypic variations in Arctic charr. For example, in charr of an Icelandic aquaculture strain, juveniles from different eggs sizes exhibit contrasting foraging behaviours (Leblanc, Benhaïm, Hansen, Kristjánsson, & Skúlason, 2011). Correlations between egg sizes and the expression of candidate genes for skeletal development were also observed in embryos and earlier juveniles of the brown Arctic charr morph from Vatnshlidarvatn (Beck et al., 2019). However, more investigations are needed to assess the importance of maternal effects on divergence in the Arctic charr of Thingvallavatn.

Finally, attention should be drawn to the role of sexual dimorphism in the ontogeny of morphological differences in Arctic charr. While this aspect has been overlook in Arctic charr, very recent work showed that the developmental changes resulting in contrasting body shapes in SB- and PL-charr occur thorough most of the juvenile period, and become moulded with sexual polymorphism at the onset of sexual maturity (De la Cámara et al., 2021). These results open the way to exciting research on the interactions of developmental mechanisms (e.g., generating trophic- and sexual polymorphism) during adaptive divergence.

1.3 Three aspects of reproductive isolation in resource polymorphism.

1.3.1 Reproductive isolation from the very beginning: a focus on behaviour.

From early on behavioural flexibility was proposed to be a basic feature of divergence by resource polymorphism, mostly considering that behaviour is more flexible than morphology (Skúlason and Smith 1995). This was not a new idea as early common-garden experiments on *Goephagus* cichlids suggested a primary role of behavioural flexibility in morphological divergence by triggering phenotypic plasticity through diet changes (Wimberger, 1992). This phenomenon was later demonstrated in studies on Arctic charr (Adams, Woltering, & Alexander, 2003). In the last two decades another aspect of behaviour, animal personality (*i.e.*, the consistent behavioural variations among individuals across time and contexts) has galvanized research in ecology and evolution. (Réale, Reader, Sol, McDougall, & Dingemanse, 2007; Roche, Careau, & Binning, 2016). Striking examples of the ecological relevance of animal personality are seen in pumpkinseed sunfish (*Lepomis gibbosus*) populations composed of individuals with different propensities to risk-taking behaviour ("shy" and "bold") segregating between littoral and open water habitats (Coleman and Wilson 1998; Wilson 1998). Animal personality may be linked to individual specialisation, with various consequences for community structures that have been reviewed elsewhere (Biro & Stamps, 2008; Wolf & Weissing, 2012). Several authors have gone further by suggesting that animal personality may facilitate speciation by inducing habitat segregation, temporal isolation and immigrant inviability, among others (Holtmann, Santos, Lara, & Nakagawa, 2017; Ingley & Johnson, 2014). Personality traits are also often genetically determined, can be highly heritable (Bell, Hankison, & Laskowski, 2009) or may also be related to stochastic developmental processes (Bierbach, Laskowski, & Wolf, 2017). But the evolutionary potential of animal personality acquires further relevance when considering that personality traits are often correlated (*i.e.*, constitute personality syndromes; Sih et al. 2012; Royauté, Hedrick, and Dochtermann 2020) and are linked to other characters like metabolism, life-history strategies or morphology (Biro & Stamps, 2008; Goodchild, Schmidt, & Durant, 2020; Polverino, Santostefano, Díaz-Gil, & Mehner, 2018). Personality traits are also likely to be pleiotropic with morphology, as seen in a study showing how body shape differed between zebrafish (*Danio rerio*) lines artificially selected for bold vs. shy personality traits (Kern, Robinson, Gass, Godwin, & Langerhans, 2016).

I argue that personality could affect the processes enounced in the extended verbal model of divergence by resource polymorphism. During the colonisation of a new geographic system, invaders of already different personality types segregate between habitats. Because of likely pleotropic effects between personality and morphology, polymorphism involving multiple behavioural and non-behavioural traits may readily appears, and strong extrinsic prezygotic isolation might emerge from early on (see strong selection vs. multifarious selection hypotheses; Nosil, Harmon, and Seehausen 2009). Meanwhile, the stability of ecological processes would quickly be reached because of the level of individual specialisation conferred by the segregating personality types. However, another possibility is that correlational selection in each habitat is not aligned with the trait covariance generated by pleiotropy. In such case, animal personality would impede adaptive divergence.

The Arctic charr of Thingvallavatn provides valuable opportunities to study the importance of behaviour in the evolution of reproductive isolation. Salmonids are promising models to study ecologically relevant variations in personality traits (Church & Grant, 2018), and standard behavioural studies are easy to implement in Arctic charr (Benhaïm, Skúlason, & Hansen, 2003; Joris, Dellinger, & Benhaïm, 2022; Leblanc et al., 2011). In Thingvallavatn, the complex differences in the habitats of PL- and SB-charr described in the previous section offers conditions to study divergence in personality traits in relation to pleiotropic physical characters and their evolution with regards to hybridization. This could be achieved though longitudinal studies on personality traits and their differences among morphs (through ontogeny), on their covariance with other traits and their covariance in hybrids. Such studies would be facilitated by the ease to raise embryos in common-garden condition and to quantify Arctic charr behaviour with standard tests.

1.3.2 Canalising or decanalising: the Evo-Devo of hybridisation.

Hybridization between early diverging populations has recently been scrutinized for its potential to increase phenotypic variance and to relax trait covariations, thereby influencing evolvability in the diverging populations (Guillaume & Whitlock, 2007; Seehausen et al., 2014; Selz, Lucek, Young, & Seehausen, 2014). Much knowledge was gained from studies on cichlid fishes morphology, mostly investigating transgressive segregation (the formation of extreme hybrid phenotypes) in relation to genetic architecture and selection regimes (Albertson & Kocher, 2005; Feller et al., 2020; Parsons, Son, & Albertson, 2011; Selz et al., 2014; Stelkens, Schmid, Selz, & Seehausen, 2009). More recently, some attention has also been directed towards unique trait combinations in hybrids caused by dominance among many unlinked genes (Arnegard et al., 2014; Thompson, Urquhart-Cronish, Whitney, Rieseberg, & Schluter, 2021). Overall, the novel phenotypes generated by hybridization have been claimed to contribute to extrinsic postzygotic isolation, to enable new niche colonization from transgressive hybrids, or to facilitate the divergence of parental populations by reshaping genetic constraints (Albertson & Kocher, 2001).

However, further insight into how hybridization affects evolvability can be gained by studying canalisation. Recall that canalisation is the buffering of the developmental responses to genetic and environmental changes, a classical process explaining the modulation of phenotypic variation through development (Hallgrimsson et al., 2019; Hallgrímsson, Willmore, & Hall, 2002; Pesevski & Dworkin, 2020; Wagner, Booth, & Bagheri-Chaichian, 1997). The importance of canalisation in the evolution of resource polymorphisms and their underlying molecular mechanisms are progressively being unravelled. For example, the regulation of Wnt signalling mediates ossification rate in East-African cichlids, generating variations in average phenotypes and in the variability of craniofacial shapes (Parsons, Trent Taylor, Powder, & Albertson, 2014). These differences in ossification rate might induce paedomorphic morphs with high phenotypic robustness, or generalist morphs benefiting from longer periods of environmental sensitivity to skull remodelling (Parsons et al., 2014). Evidence for widespread misexpression across the genome in hybrids of early diverging morphs, including Salmonids, is accumulating (McGirr & Martin, 2019; Renaut, Nolte, & Bernatchez, 2009a), so disruptions in the mechanism shaping canalisation during hybridization are to be expected.

Disruptions in developmental regulatory mechanisms are expected to affect developmental stability (*i.e.*, the resilience to stochastic deviations from the developmental trajectories), as observed within populations subjected to environmental stress (Lazić, Carretero, Crnobrnja-Isailović, & Kaliontzopoulou, 2014). Hence, developmental stability might be affected by gene expression misregulation in hybrids, which would generate postzygotic isolation. However, many uncoupled developmental mechanisms may intervene during the development of hybrids, and very little empirical information is available. One exception is a phenotypic study on floral traits in spurge creepers (*Dalechampia scandens*), which reported decreased phenotypic variance in hybrids of closely related populations – and increased variance in genetically distant populations – but no evidence for consistent patterns of lower developmental stability in hybrids of populations with higher genetic distances (Pélabon, Carlson, Hansen, Yoccoz, & Armbruster, 2004).

The Arctic charr of Thingvallavatn is a prime model to study the Evo-Devo consequences of hybridisation. The PL- and SB-charr, which constitute differentiated populations while overlapping in the timing and the location of spawning, are especially suitable to tackle questions on hybridization. The respective niches of the two morphs also imply distinct and complex habitat differences (e.g., in physical habitat structure, and in the availability and behaviour of prey) that may constrain the life history and development of trophic morphology through ontogeny (Sandlund et al. 1987; Antonsson 1992). These evolutionary and ecological properties provide a valuable ground to study the effects of hybridization on the evolution of trait covariances. Furthermore, canalisation has shaped phenotypic variability in the Arctic charr of Thingvallavatn, which includes trophic traits and may differ in magnitude between morphs (Küttner et al., 2014, 2013; Parsons, Sheets, et al., 2011). Thorough investigations of the regulatory mechanisms underlying phenotypic variability (and thereby evolvability) are achievable thanks to the ease to study the development of ecologically relevant traits in charr reared in common garden conditions (De la Cámara et al., 2021; Eiríksson et al., 1999; Kapralova et al., 2015; Ponsioen, 2020). Furthermore, the molecular mechanisms underlying the development of phenotypic variation are now accessible from the genomic and bioinformatic resources on salmonids accumulated over the past years. Relevant regulatory pathways and candidate genes related to trophic trait differentiation in the four charr morph and in other Icelandic Arctic charr populations have now been identified (Ahi et al., 2013, 2014, 2015; Beck et al., 2019). Furthermore, the whole transcriptomes of PL-, LB- and SB-charr have been sequenced during the development of most cartilage precursors of cranial bones (Guðbrandsson et al., 2019), and two reference genomes of *Salvelinus sp*. are now available (Christensen et al. 2018; Smith et al. 2022).

1.3.3 Matching habitat choice and the integration of ecology, ontogeny and premating isolation.

An idea worth considering in relation to trophic polymorphism is that animals can select habitats yielding the best fitness prospects according to their phenotype (Camacho & Hendry, 2020; Edelaar et al., 2019; Holtmann et al., 2017). In that way, this phenomenon called matching habitat choice is antithetical to phenotypic plasticity since the former involves individuals changing their environment rather than their phenotype. For example, imagoes of azure sand grasshoppers (*Sphingonotus azurescens*, known for body colour variations acquired by plasticity during earlier nymphal stages) segregate between habitats

in which they are most cryptic when colonizing novel environments (*i.e.*, urban pavements ; Edelaar et al., 2019). Matching habitat choice predicts that beside being selective targets, individuals are selective agents *(i.e.*, choosing habitat with different selection regime). This phenomenon has been proposed to induce spatial segregation among genotypes, thereby facilitating local adaptations and impeding gene flow (Edelaar et al., 2019; Edelaar, Siepielski, & Clobert, 2008; Holtmann et al., 2017). I suggest that matching habitat choice may hasten divergence not only by generating spatial isolation for breeding, but also by subjecting their offspring to contrasting environmental conditions.

Salmonids are ideal models to investigate the relevance of habitat matching during speciation. Many salmonids encounter highly heterogeneous environments when spawning and have evolved elaborate forms of habitat selection. For instance, bull trout (*Salvelinus confluentus*) select areas with high temperature and water discharge for spawning (Baxter & Mcphail, 1999). Similarly, spawning sites of brook charr (*Salvelinus fontinalis*) are characterised by upwelling groundwater flow offering specific conditions of oxygen concentration and conductivity (Guillemette et al., 2011). Some evidence for matching habitat choice have even been reported in sockeye salmon (*Oncorhynchus nerka*), as females in age classes that were the most vulnerable to predation from bears appeared to spawn in the deepest waters (Camacho & Hendry, 2020; Hendry, Berg, & Quinn, 2001). Furthermore, salmonids typically exhibit strong breeding site fidelity across generations that have a high potential to generate population structures (Adams et al. 2006; Kapralova et al. 2011), increasing the relevance of habitat choice matching as a driver of pre-mating reproductive isolation. But of primary importance is the proposition that the ontogeny of salmonid embryos and newly hatched juveniles is tightly linked to local environmental factors. While temperature and oxygen concentration dictate developmental rates (Gorodilov, 1996; Quinn, 2018), food availability at early post-hatching stages can affect growth, which may induce long-lasting physiological consequences (Metcalfe & Monaghan, 2001; Nicieza & Metcalfe, 1997) and, considering the relationship between early size and foraging behaviour (Benhaïm et al., 2003), can have indirect effects on ecologically relevant behavioural variation.

The Arctic charr of Thingvallavatn constitutes an exceptional model to study the interplay of habitat choice and development in a context of divergence. Notably, one morph, the LSB-charr, spawns in from mid-July to mid-August, which is unusual for Arctic charr (Skúlason, Snorrason, et al., 1989). The LB-charr is therefore reproductively isolated from the other three morphs spawning in September-December (Skúlason, Snorrason, et al., 1989). The best known spawning site of LB-charr differs from known spawning sites of the other morphs in being strongly influenced by the inflow of cold springs (Skúlason, Snorrason, et al., 1989). The cold springs have the effect of keeping temperature of the LB-spawning site colder during the summer months and into the autumn (June – October) but slightly warmer during the winter months (January – April; Skúlason, Snorrason, et al., 1989). Because of this temperature regime, the spawning site of the LB-charr also appears to maintain the activity of important benthic prey such as chironomids through the winter months (Sandlund et al., 1988). LB-charr also depend heavily on the snail *Radix peregra* for food during the active season (mid-May to November; Malmquist et al. 1992). Adult LB-charr prefer large snails which are at high densities in May and early June when they reproduce and die. Thus the timing of reproduction of the snail may be crucial in facilitating the early maturation of gonads in the LB-charr (Snorrason, 2000). These ecological factors may have enabled the

evolution of either or both of the remarkable spawning timing of LB-charr and the fast growth of their offspring (Skúlason, Snorrason, et al., 1989). All these factors provide an ideal "field laboratory" to gain further insight on choice of spawning habitat and its ontogenetic consequences.

The ease to conduct field studies on spawning LB-charr (Sigurjónsdóttir & Gunnarsson, 1989) also makes it a prime model to study habitat choice. First, conspicuous visual cues of spawning site selection are left by females cleaning the substrate from debris, silt, and algae when establishing their nest (redd). Second, LB-charr spawn on the easily accessible littoral zone of the lake, and unlike the three other morphs, have diurnal spawning behaviour and do not appear to be disturbed by human observers. This enables direct observations of behavioural proxies of spawning site competition, which are stereotypic behaviours exhibited by salmonids during courtship and agonistic interactions (Esteve, 2005; Fabricius, 1953; Sigurjónsdóttir & Gunnarsson, 1989). Therefore, together with the opportunities to conduct ontogenetic studies enounced in the previous sections, the mating behaviour of the LB-charr enables provides promising resources to investigate the role of matching habitat choice within the extended view of the resource polymorphism.

2 Aims of the thesis.

This thesis combines five studies attempting to characterise reproductive isolation in the Arctic charr of Thingvallavatn and to unravel the underlying ecological, evolutionary and developmental processes (Table 1). While I am the lead authors of the five papers, many collaborators were involved. Therefore, I hereafter refer to me and my co-authors with the first person of the plural.

The first four papers focus on the PL- and the SB-charr, the two morphs constituting phenotypically and genetically distinct populations despites large putative spatiotemporal overlaps during spawning. First, we investigated how the covariance structure of multiple traits influence phenotypic divergence between the two morphs, and how this may be affected by hybridization (Paper I). Second, we extended this line of thought to personality traits and behavioural syndromes in SB-and PL-charr and their hybrids (Paper II). Third, we dove into the molecular mechanisms behind postzygotic isolation and the effects of hybridisation on evolvability through gene expression studies (Paper III). Fourth, we established a synthesis of the mechanisms of reproductive isolation between PL- and SB-charr through a comprehensive study on spatiotemporal isolation, assortative mating, incompatibilities to fertilisation and postzygotic isolation (Paper IV).

The last study focuses on the LB-charr and is a primer on how combined investigation on behaviour and ontogeny can lead to a better understanding of the interplay of habitat choice, reproductive isolation and development within theoretical models of resource polymorphism (Paper V).

Table 1. *The main research question of each paper and its context within the Eco-Evo-Devo framework[1].*

Question	Context and processes			Biological Model	Paper
	Devo	***Evo***	***Eco***		
1. What are the evolutionary consequences of hybridisation during speciation?	Developmental processes underlying evolvability (canalisation, developmental stability) are relaxed	Divergence impeded as trait integration or canalisation does not progress vs. divergence facilitated as introgressed populations benefit from new phenotypic variation and/or hybrids with less stable development are selected against	New niches are colonized by extreme phenotypes Communities more or less defined as biological variation within morphs determine their efficiency as "ecological species". Henceforth, selection regimes are more or less stable.	Offspring of PL-charr, SB-and hybrids in common garden conditions	**Papers I to III**
2. What is the importance of animal personality in adaptive divergence?	Different personality traits readily occurring within populations and pleiotropic with morphology and physiology.	Divergence facilitated/impeded as individual from populations with integrated traits segregate between habitats vs. trait covariance evolving rapidly	Same as in line 1	Same as in line 1	**Paper II**
3. How are the molecular mechanisms shaping phenotypic variation affected during speciation?	The rapid evolution of gene expression drives differentiation in canalisation and developmental stability. Disruption of gene expression during hybridisation affect the development of phenotypic variation	Same as in line 1	Same as in line 1	Same as in line 1	**Paper III**
4.Which reproductive barriers evolve early in divergence?	Developmental deficiencies in hybrids from genetic incompatibilities Development of intermediate or	Mate choice generates pre-zygotic barriers Differences in the ecology of diverging populations result in temporal and spatial isolation	Same as in line 1, and: Selection regimes induce postzygotic isolation Ecological opportunities enable new colonisation of novel phenotypes from hybridisation	Same as in line 1	**Paper IV**

	transgressive phenotypes in hybrids	Evolution of postzygotic isolation and possible reinforcement from mate choice.	Selection regimes interfere with sexual selection		
5. What is the importance of habitat choice during divergence?	Variations in abiotic parameters during development shapes phenotypic diversity in the offspring	Breeding environment fidelity and matching habitat choice the rapid establishment of contrasting phenotypes. Meanwhile, spatial patterns in in the distribution of genotypes arise (spatial isolation).	Habitat complexity affects the evolution of habitat choice.	Wild LB-charr over a spawning site	**Paper V**

[1] *Hypothetical Eco, Evo and Devo processes, not exhaustive.*

3 Methods.

3.1 Postzygotic isolation and Evo-Devo of hybridization in PL- and SB-charr (Papers I to IV).

We studied the ontogeny of PL- and SB-charr and their hybrids through common-garden experiments. These methods have been used multiple times in the Arctic charr of Thingvallavatn and have enabled successful characterisations of genetic-bases for variations in morphology (De la Cámara et al., 2021; Eiríksson et al., 1999; Kapralova et al., 2015; Parsons, Sheets, et al., 2011; Ponsioen, 2020; Skúlason, Noakes, et al., 1989), foraging behaviour (Skúlason et al., 1993), life-history (Skúlason et al., 1996) and gene expression (Ahi et al., 2015; Guðbrandsson et al., 2016).

For Papers I and II, and parts of Paper III and IV, we conducted experiments on Arctic charr offspring reared in a single common-garden setup. The workflow is illustrated in Figure 4. The offspring reared in the setup originated from wild specimens collected at the spawning grounds. At hatching, about 200 free-swimming embryos were placed into separate containers to collect individual-level data throughout ontogeny (for Papers I and II).

In Papers II, we collected longitudinal measurements of morphology, growth, ontogenetic shifts and yolk-sac resorption in the individually reared specimens. We did so by taking photographs of the specimens at four developmental time points (from hatching to several months into exogeneous feeding). We also conducted feeding experiments following the methods of previous studies on behavioural variations in early Arctic charr juveniles (Leblanc et al., 2011). These measurements enabled us not only to test for differences in average trait values, variances and covariances between crosses types, but also to assess covariance between traits developing at different ontogenetic time points. We analysed most of these data altogether by generating phenotypic matrices of variance–covariance (**P** matrix) of the PL-, the SB- and the hybrid offspring. We then compared the cross types based on their **P** matrix properties with methods from the field of quantitative genetics (Aguirre, Hine, McGuigan, & Blows, 2014; A. G. Jones, Arnold, & Bürger, 2003; Krzanowski, 1979). We also compared the cross types using geometric morphometrics (Adams, Rohlf, & Slice, 2013; Rohlf & Corti, 2000) to assess the covariance between the univariate variables in **P** and the multivariate head shape data.

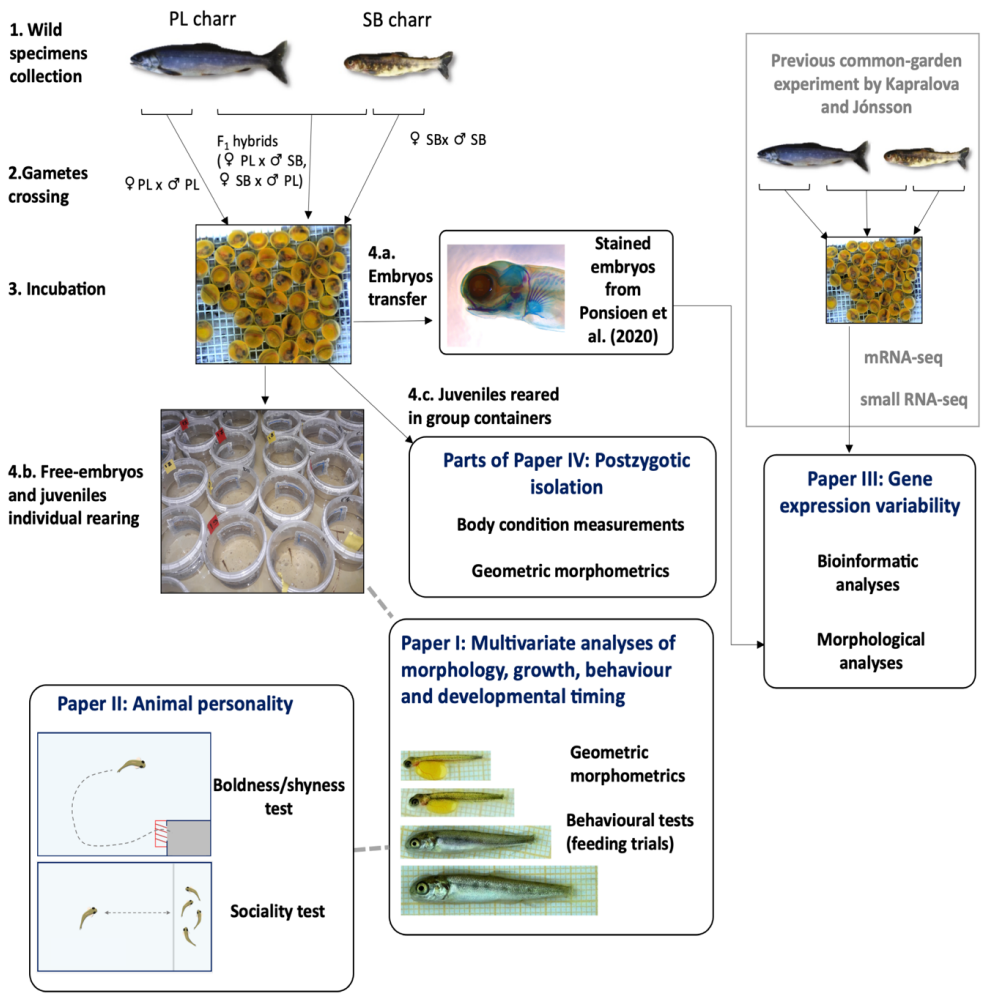


Figure 4. *Workflow and main experiments of the studies on phenotype divergence and hybridization in the PL- and the SB-charr (Papers I to IV). Paper IV is based on the data collected along the whole workflow and during other studies (see text).*

In paper II, we studied personality traits in the specimens reared individually. We conducted two types of experimental tests when the juveniles reached two months after the onset of exogenous feeding. The first test consisted in assessing the position of each individual along a boldness/shyness axis. This was achieved through measurements of activity (distance moved), thigmotaxis (the aversion for locations away from vertical surfaces) and/or the time taken to exit a shelter. We did so by tracking each fish in an open arena with a shelter (open-field test), a method that has proven efficient for boldness/shyness measurement in fishes, including Arctic charr (Benhaïm, Ferrari, Chatain, & Bégout, 2016; Dahlbom, Lagman, Lundstedt-Enkel, Sundström, & Winberg, 2011; Kern et al., 2016; Joris et al., 2022). Second, we measured in the same specimens the propensity to interact with a shoal of conspecific

(sociality). We tracked each individual in an arena with a glass compartment containing other charr juveniles. This setup already prove successful to quantify sociality in other teleost species (Benhaïm et al., 2016). This way, we could compare the cross types using estimate of average trait values, individual consistency (personality *per se*), within group variance and trait correlations (personality syndromes).

In Paper III we conducted gene expression analyses on biological material from a previous common-garden experiment (Figure 4). This experiment involved the same crossing design as in the setup for Papers I and II (PL- and SB-offspring and F_1 hybrids). Whole embryos were sampled at two developmental time points: when the first cartilage precursors of cranial bones appear and when most of cartilaginous structures of the cranial and the pharyngeal skeletons are formed, respectively. The samples were sequenced for mRNA and small noncoding RNAs (microRNAs). From thereon, we performed differential expression analyses. However, the most innovative part of our project stems from our estimations of gene expression variability as a proxy of canalization. We compared the cross types according to their profile of gene expression variability, which we generated by adapting algorithms developed for medical sciences. Finally, we assessed the phenotypic relevance of gene expression patterns in candidate genes for lower jaw development (*Bmp4* and *patched1*) through morphological analyses of embryos with stained cartilage and bones, which we sampled from the rearing setup described for Paper I and II.

3.2 Reproductive barriers in PL- and SB-charr (Paper IV)

We estimated the importance of reproductive barriers through a fishing survey, a mate choice experiment and the data for the common-garden experiments of Paper I and II. The fishing survey consisted in laying nets weekly overnight and at two known spawning sites. The collected specimens were then dissected to assess the degree of gonadal ripeness.

Assortative mating was another possible prezygotic barrier, which we studied in an experiment confronting one SB- or PL-female with two males, one of each morph. Video records from this experiment enabled quantifying spawning events and courtship events involving each male as proxies of mate choice.

We assessed the importance of post-mating barriers (fertilisation success and postzygotic isolation) by using the data from the common-garden experiment in Papers I and II. These data enabled assessing the fertilisation successes and pre-hatching embryo survival in the different cross types. Then, we collect biometric data to estimate growth on a set of specimens that were collected after hatching or reared in group container and sampled several months after the onset of the exogeneous feeding containers. After the experiment was shut down, we dissected the specimens to estimate body condition (though relative body weight and relative wet liver weight) as cues of postzygotic isolation. We also conducted morphometric analyses on the same specimens to assess both body condition and body shape variations (as a potential signal of extrinsic postzygotic isolation).

Finally, we combined all these data to calculate indices of total reproductive isolation and of the relative strength of each reproductive barrier. We did these calculations by adapting standard methods from the field of speciation research (Coyne & Orr, 1997; Ramsey, Bradshaw, & Schemske, 2003; Rolan-Alvarez & Caballero, 2000; Sobel & Chen, 2014).

3.3 Evo-Devo of habitat choice in the LB-charr (Paper V)

We investigated spawning habitat preferences in LB-charr and their consequences for embryo development by conducting field studies and data analyses from a previous common-garden experiment. We expected heterogeneous temperature conditions over the best studied spawning ground of LB-charr, and that LB-charr have evolved habitat selection toward sites offering the best conditions for embryos development. Evidence for habitat selection would be observed as unequal distribution of redds (charr "nests") across the spawning site, higher fish densities, and strong competitions above some redds. First, we used a drone survey to assess the spatial heterogeneity of redds over the best know LB-spawning site. Meanwhile, we collected video records and continuous temperature measurements in redd scattered over the spawning site. By analysing the video records, we estimated female condition, male densities, aggression and courtship behaviours as proxies of site selection. Then, we inferred the developmental rates of the offspring in the monitored redds using the temperature records, by following standard calculations for salmonids (Gorodilov, 1996; Quinn, 2018). Based on these inferences and on data from the common-garden experiment of Paper I and II, we estimated timing variations in hatching and in the onset of exogeneous feeding in relation to temperature over the spawning ground. We visualised the variations in developmental rate among redds by using stained embryos from Kapralova et al. (2015).

4 Summary of the papers.

In Paper I, we conducted an examination of the traits putatively involved in the divergence of the PL- and the SB-charr. We then assessed covariance between these traits and how it would affect the phenotype of F_1 hybrids. These traits were measured along ontogeny and involved growth, yolk sac resorption, developmental timing (hatching and the onset of exogeneous feeding), head morphology and feeding behaviour. Growth trajectories provided the strongest signal of phenotypic divergence between the two charr. Strikingly, the hybrids did not show intermediate nor delayed growth but were similar to the smallest morph, suggesting parental biases in the inheritance of growth patterns. However, we did not observe extensive differences in trait covariance between the two morphs and their hybrids. Growth was linked to head morphology (suggesting that morphological variations in early juveniles relate to simple allometric effects) but this was the only strong signal of covariance observed between all the measured traits. Furthermore, we did not report evidence for differences in overall phenotypic variance between morphs, nor for enhanced phenotypic variability in their hybrids.

In Paper II, we assessed whether and how PL- and SB-juveniles show genetically based differences in personality that conform to their respective ecological niches, and whether these differences could contribute to reproductive isolation by generating maladaptive behaviours in hybrids. Studying three aspects of behavioural variation (average trait value, consistent individual differences and behavioural syndromes), we assessed the sociality and risk-taking propensity of hybrids and pure-morph offspring reared in common-garden conditions. While no difference in average behavioural responses could be observed, the hybrids tended to show less repeatable behaviours and were not intermediate for behavioural syndromes that appear to differ between the two morphs. These results provide limited evidence of personality trait divergence in the two charr, and suggest subtle, nonadditive effects of hybridization on the development of the studied personality traits.

In paper III, we tested whether SB- and PL-charr differ in gene expression variability during development. In this study, we also investigated how dominance would affect gene expression in hybrids. Through a common-garden experiment, we identified clusters of genes with covarying expression variability that differed between the two morphs. In the hybrids, gene expression variability was substantially affected by maternal effects, biases towards the PL-charr, and to some extent by over- and underdominance. The expression variability profiles were consistent for mRNA and miRNA datasets. The complex dominance patterns also concerned candidate genes involved in the lower jaw development, but were only partially reflected by morphological variations. In all, our results from Paper III suggest that gene expression variability can evolve rapidly among sympatric populations, and that the effects of hybridization on phenotypic variability can be altered or buffered by many developmental pathways.

Paper IV is a summary paper of reproductive isolation between the PL- and the SB-charr. We estimated the importance of reproductive barriers with data from a fishing survey, a mate choice experiment and a common-garden experiments involving the offspring of each morph and F_1 hybrids. Differences in the timing and location of spawning accounted from most of the estimated reduction in gene flow. However, this barrier was ineffective in the SB-charr, for which assortative mating and postmating isolation (fertilization failures and/or embryo mortality) tended to result in partial reproductive isolation.

In Paper V, we focused on the spawning behaviour of LB-charr and its consequences on embryo development. We studied spawning habitat choice with aerial surveys, behavioural observations, and temperature monitoring. We also relied on a rearing experiment to assess the effects of spawning habitat choice (variations in temperature conditions) on the developmental rate of the offspring. Aerial footage revealed that most nests (redds) were established in shallow parts of the spawning area. The behavioural observations also suggested stronger male–male competition and more intense courtship behaviours in shallow redds. While water depth did not correlate with temperature at the time of spawning, the temperatures recorded at the shallow redds were consistently lower in the two months following the video recordings, likely because of the proximity of cold springs. Laboratory experiments demonstrated that the temperature regimes in shallow waters can delay hatching by about a month, likely impacting the timing of the onset of exogeneous feeding in the offspring.

5 Conclusions and future directions.

Our studies of phenotypic differences in PL- and SB-charr and their hybrids show that developmental biases may not affect adaptive divergence in the two morphs, even though multiple traits are likely involved. Likewise, hybridization may have little impact on the evolvability of the two morphs. However, studying gene expression yielded more substantial information about the role of development in evolvability via the regulation of gene expression variability. Moreover, the dominance patterns of gene expression variability in hybrids indicated that hybridization may increase phenotypic variations in biased directions of the phenotype space.

Our summary study on reproductive barriers between PL- and SB-charr is in the line with other evidence of the rapid build-up of spatial and temporal isolation during breeding. However, our results also suggest that postzygotic isolation might evolve early during divergence contrary to general expectations.

Altogether, the sections of this thesis focusing PL- and SB-charr constitute a rare integrative insight into the multiple aspects of reproductive isolation within a single system. Hence, the Arctic charr of Thingvallavatn, which was already an ideal candidate to studies on adaptive divergence, now stands as model with established knowledge on the incidence of different reproductive barriers, the effects of hybridization and the developmental aspects of speciation. This updated view will hopefully motivate further work on the importance of development and behaviour in speciation. Furthermore, investigations with larger sample sizes and focusing on postglacial fishes at different point along the speciation continuum would provide a much-needed insight onto the evolution of reproductive isolation within the Eco-Evo-Devo framework.

Finally, our study on spawning behaviour in LB-charr has refined the idea that habitat choice during spawning may have profound consequences on phenotypic diversity by altering offspring development. I hope that our paper will motivate confirmatory studies, but also encourage further work to establish whether the observed patterns of spawning site selection are induced by matching habitat choice. Further assessment of the fitness outcome of habitat choice on the offspring will be of great interest. The consequences of such developmental rate modulations may for example affect the synchrony between ontogenetic shifts and prey seasonality. However, a wider range of ontogenetic and evolutionary consequence explored in the former chapters of the thesis (when integrating the interplay of behavioural variation, developmental time and morphology) is also expected, setting habitat choice in a very dynamic Eco-Evo-Devo view. The matching habitat choice hypothesis is still in its infancy and much remains to be explored regarding its evolutionary relevance. Therefore, very exciting discoveries on the interplay of matching habitat choice and the evolution of reproductive isolation are to be anticipated.

References.

Abbott, R., Albach, D., Ansell, S., Arntzen, J. W., Baird, S. J. E., Bierne, N., … Zinner, D. (2013). Hybridization and speciation. *Journal of Evolutionary Biology, 26*(2), 229–246. https://doi.org/10.1111/j.1420-9101.2012.02599.x

Ackermann, R. R., Rogers, J., & Cheverud, J. M. (2006). Identifying the morphological signatures of hybridization in primate and human evolution. *Journal of Human Evolution, 51*(6), 632–645. https://doi.org/10.1016/j.jhevol.2006.07.009

Adalsteinsson, H., Jónasson, P. M., & Rist, S. (1992). Physical characteristics of Thingvallatn, Iceland. *Oikos, 64*, 121–135.

Adams, C. E., Hamilton, D. J., Mccarthy, I., Wilson, A. J., Grant, A., Alexander, G., … Skúlason, S. (2006). Does breeding site fidelity drive phenotypic and genetic sub-structuring of a population of arctic charr? *Evolutionary Ecology, 20*(1), 11–26. https://doi.org/10.1007/s10682-005-2489-4

Adams, C. E., Woltering, C., & Alexander, G. (2003). Epigenetic regulation of trophic morphology through feeding behaviour in Arctic charr, *Salvelinus alpinus*. *Biological Journal of the Linnean Society*, *78*(1), 43–49. https://doi.org/10.1046/j.1095-8312.2003.00126.x

Adams, D. C., Rohlf, F. J., & Slice, D. E. (2013). A field comes of age: Geometric morphometrics in the 21st century. *Hystrix, 24*(1), 7–14. https://doi.org/10.4404/hystrix-24.1-6283

Aguirre, J. D., Hine, E., McGuigan, K., & Blows, M. W. (2014). Comparing G: Multivariate analysis of genetic variation in multiple populations. *Heredity, 112*(1), 21–29. https://doi.org/10.1038/hdy.2013.12

Ahi, E. P., Guðbrandsson, J., Kapralova, K. H., Franzdóttir, S. R., Snorrason, S. S., Maier, V. H., & Jónsson, Z. O. (2013). Validation of reference genes for expression studies during craniofacial development in Arctic Charr. *PLoS ONE, 8*(6), e66389. https://doi.org/10.1371/journal.pone.0066389

Ahi, E. P., Kapralova, K. H., Pálsson, A., Maier, V. H., Guðbrandsson, J., Snorrason, S. S., … Franzdóttir, S. R. (2014). Transcriptional dynamics of a conserved gene expression network associated with craniofacial divergence in Arctic charr. *EvoDevo, 5*(1), 1–19. https://doi.org/10.1186/2041-9139-5-40

Ahi, E. P., Steinhäuser, S. S., Pálsson, A., Franzdóttir, S. R., Snorrason, S. S., Maier, V. H., & Jónsson, Z. O. (2015). Differential expression of the aryl hydrocarbon receptor pathway associates with craniofacial polymorphism in sympatric Arctic charr. *EvoDevo, 6*(1), 1–18. https://doi.org/10.1186/s13227-015-0022-6

Albertson, R. C., & Kocher, T. D. (2001). Assessing morphological differences in an adaptive trait: A landmark-based morphometric approach. *Journal of Experimental*

Zoology, 289(6), 385–403. https://doi.org/10.1002/jez.1020

Albertson, R. C., & Kocher, T. D. (2005). Genetic architecture sets limits on transgressive segregation in hybrid cichlid fishes. *Evolution, 59*(3), 686–690. https://doi.org/10.1111/j.0014-3820.2005.tb01027.x

Antonsson, Ú. (1992). The Structure and Function of Zooplankton in Thingvallavatn, Iceland. *Oikos, 64*(1), 188–221. https://doi.org/10.2307/3545052

Arnegard, M. E., McGee, M. D., Matthews, B., Marchinko, K. B., Conte, G. L., Kabir, S., … Schluter, D. (2014). Genetics of ecological divergence during speciation. *Nature, 511*(7509), 307–311. https://doi.org/10.1038/nature13301

Baxter, J. S., & Mcphail, J. D. (1999). The influence of redd site selection , groundwater upwelling , and over-winter incubation temperature on survival of bull trout (*Salvelinus confluentus*) from egg to alevin. *Canadian Journal of Zoology, 77*(8), 1233–1239.

Beck, S. V, Räsänen, K., Ahi, E. P., Kristjánsson, B. K., Skúlason, S., Jónsson, Z. O., & Leblanc, C. A. (2019). Gene expression in the phenotypically plastic Arctic charr (*Salvelinus alpinus*): A focus on growth and ossification at early stages of development. *Evolution & Development, 21*(1), 16–30. https://doi.org/10.1111/ede.12275

Bell, A. M., Hankison, S. J., & Laskowski, K. L. (2009). The repeatability of behaviour: a meta-analysis. *Animal Behaviour, 77*(4), 771–783. https://doi.org/10.1016/j.anbehav.2008.12.022

Benhaïm, D., Ferrari, S., Chatain, B., & Bégout, M. L. (2016). The shy prefer familiar congeners. *Behavioural Processes, 126*(6), 113–120. https://doi.org/10.1016/j.beproc.2016.03.008

Benhaïm, D., Skúlason, S., & Hansen, B. R. (2003). Behavioural variation in juvenile Arctic charr in relation to body size. *Journal of Fish Biology, 62*(6), 1326–1338. https://doi.org/10.1046/j.1095-8649.2003.00114.x

Bhullar, B.-A. S., Marugán-Lobón, J., Racimo, F., Bever, G. S., Rowe, T. B., Norell, M. A., & Abzhanov, A. (2012). Birds have paedomorphic dinosaur skulls. *Nature, 487*(7406), 223–226. https://doi.org/10.1038/nature11146

Bierbach, D., Laskowski, K. L., & Wolf, M. (2017). Behavioural individuality in clonal fish arises despite near-identical rearing conditions. *Nature Communications, 8*(1), 1–7. https://doi.org/10.1038/ncomms15361

Biro, P. A., & Stamps, J. A. (2008). Are animal personality traits linked to life-history productivity? *Trends in Ecology and Evolution, 23*(7), 361–368. https://doi.org/10.1016/j.tree.2008.04.003

Brachmann, M. K., Parsons, K., Skúlason, S., & Ferguson, M. M. (2021). The interaction of resource use and gene flow on the phenotypic divergence of benthic and pelagic morphs of Icelandic Arctic charr (*Salvelinus alpinus*). *Ecology and Evolution*, *11*(12), 7315-7334. https://doi.org/10.1002/ece3.7563

Camacho, C., & Hendry, A. P. (2020). Matching habitat choice: it's not for everyone. *Oikos, 129*(5), 689–699. https://doi.org/10.1111/oik.06932

Christensen, K., Rondeau, E., Minkley, D., Leong, J., Nugent, C., Danzmann, R., … Koop BF. (2018). The Arctic charr (*Salvelinus alpinus*) genome and transcriptome. *PloS one, 13*(9), https://doi.org/10.1371/journal.pone.0204076

Church, K. D. W., & Grant, J. W. A. (2018). Does increasing habitat complexity favour particular personality types of juvenile Atlantic salmon, *Salmo salar*? *Animal Behaviour, 135*, 139–146. https://doi.org/10.1016/j.anbehav.2017.11.006

Coleman, K., & Wilson, D. S. (1998). Shyness and boldness in pumpkinseed sunfish: Individual differences are context-specific. *Animal Behaviour, 56*(4), 927–936. https://doi.org/10.1006/anbe.1998.0852

Coyne, J. A., & Orr, H. A. (1997). "Patterns of speciation in *Drosophila*" revisited. *Evolution, 51*(1), 295–303. https://doi.org/10.1111/j.1558-5646.1997.tb02412.x

Dahlbom, S. J., Lagman, D., Lundstedt-Enkel, K., Sundström, L. F., & Winberg, S. (2011). Boldness predicts social status in zebrafish (*Danio rerio*). *PLoS ONE, 6*(8), 2–8. https://doi.org/10.1371/journal.pone.0023565

Danzmann, R. G., Ferguson, M. M., Skúlason, S., Snorrason, S. S., & Noakes, D. L. G. (1991). Mitochondrial DNA diversity among four sympatric morphs of Arctic charr, *Salvelinus alpinus L.*, from Thingvallavatn, Iceland. *Journal of Fish Biology, 39*(5), 649–659.

De la Cámara, M. (n.d.). Evolutionary history of the Arctic charr in Thingvallavatn.

De la Cámara, M., Ponsioen Lieke, Horta-Lacueva, Q. J. B., & Kapralova, K. H. (2021). The dynamic ontogenetic patterns of adaptive divergence and sexual dimorphism in Arctic charr. BioRxiv, 2021.01.15.426104. Retrieved from https://doi.org/10.1101/2021.01.15.426104

Dobzhansky, T. (1936). Studies on hybrid sterility - II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. *Genetics, 21*(2), 169–223. https://doi.org/10.1007/BF00374056

Doenz, C. J., Bittner, D., Vonlanthen, P., Wagner, C. E., & Seehausen, O. (2018). Rapid buildup of sympatric species diversity in Alpine whitefish. *Ecology and Evolution, 8*(18), 9398–9412. https://doi.org/10.1002/ece3.4375

Edelaar, P., Baños-villalba, A., Quevedo, D. P., Escudero, G., Bolnick, D. I., Jordán-andrade, A., & Baños-villalba, A. (2019). Biased movement drives local cryptic coloration on distinct urban pavements. *Proceedings of the Royal Society B, 286*(1912), 20191343. https://doi.org/http://dx.doi.org/10.1098/rspb.2019.1343

Edelaar, P., Siepielski, A. M., & Clobert, J. (2008). Matching habitat choice causes directed gene flow: A neglected dimension in evolution and ecology. *Evolution, 62*(10), 2462–2472. https://doi.org/10.1111/j.1558-5646.2008.00459.x

Eiríksson, G. M., Skúlason, S., & Snorrason, S. S. (1999). Heterochrony in skeletal development and body size in progeny of two morphs of Arctic charr from Thingvallavatn, Iceland. *Journal of Fish Biology*, *55*(1999), 175–185. https://doi.org/10.1111/j.1095-8649.1999.tb01054.x

Esteve, M. (2005). Observations of spawning behaviour in Salmoninae: *Salmo*, *Oncorhynchus* and *Salvelinus*. *Reviews in Fish Biology and Fisheries, 15*(1–2), 1–21. https://doi.org/10.1007/s11160-005-7434-7

Fabricius, E. (1953). Aquarium observations on the spawning behaviour of the char, *Salmo alpinus*. *Rep. Inst. Freshwater. Res. Drottningholm, 37*, 14–48.

Feller, A. F., Selz, O. M., McGee, M. D., Meier, J. I., Mwaiko, S., & Seehausen, O. (2020). Rapid generation of ecologically relevant behavioral novelty in experimental cichlid hybrids. *Ecology and Evolution, 10*(14), 7445–7462. https://doi.org/10.1002/ece3.6471

Foote, A. D. (2017). Sympatric Speciation in the Genomic Era. *Trends in Ecology and Evolution, 33*(2), 85–95. https://doi.org/10.1016/j.tree.2017.11.003

Franklin, O. D., Skúlason, S., Morrissey, M. B., & Ferguson, M. M. (2018). Natural selection for body shape in resource polymorphic Icelandic Arctic charr. *Journal of Evolutionary Biology, 31*(10), 1498–1512. https://doi.org/10.1111/jeb.13346

Gilbert, S. F., Bosch, T. C. G., & Ledón-Rettig, C. (2015). Eco-Evo-Devo: Developmental symbiosis and developmental plasticity as evolutionary agents. *Nature Reviews Genetics, 16*(10), 611–622. https://doi.org/10.1038/nrg3982

Goodchild, C. G., Schmidt, L. M., & Durant, S. E. (2020). Evidence for the 'behavioural character' hypothesis : does boldness programme disparate antipredator strategies ? *Animal Behaviour*, *164*, 123–132. https://doi.org/10.1016/j.anbehav.2020.04.010

Gorodilov, Y. N. (1996). Description of the early ontogeny of the Atlantic salmon, *Salmo salar*, with a novel system of interval (state) identification Yuriy. *Environmental Biology of Fishes, 47*, 109–127. https://doi.org/10.1080/08828032.1987.10390235

Guðbrandsson, Johannes, Ahi, E. P., Franzdóttir, S. R., Kapralova, K. H., Kristjánsson, B. K., Steinhaeuser, S. S., … Pálsson, A. (2016). The developmental transcriptome of contrasting Arctic charr (*Salvelinus alpinus*) morphs. *F1000Research, 4*, 136. https://doi.org/10.12688/f1000research.6402.2

Guðbrandsson, Jóhannes, Franzdóttir, S. R., Kristjánsson, B. K., Ahi, E. P., Maier, V. H., Kapralova, K. H., … Pálsson, A. (2018). Differential gene expression during early development in recently evolved and sympatric Arctic charr morphs. *PeerJ, 6*. https://doi.org/10.7717/peerj.4345

Guðbrandsson, Jóhannes, Kapralova, K. H., Franzdóttir, S. R., Bergsveinsdóttir, T. M., Hafstað, V., Jónsson, Z. O., … Pálsson, A. (2019). Extensive genetic divergence between recently evolved sympatric Arctic charr morphs. *Ecology and Evolution, 9*(9), 1–20. https://doi.org/10.1101/489104

Guillaume, F., & Whitlock, M. C. (2007). Effects of migration on the genetic covariance

matrix. *Evolution, 61*(10), 2398–2409. https://doi.org/10.1111/j.1558-5646.2007.00193.x

Hallgrimsson, B., Green, R. M., Katz, D. C., Fish, J. L., Bernier, F. P., Roseman, C. C., … Marcucio, R. S. (2019). The developmental-genetics of canalization. *Seminars in Cell and Developmental Biology, 88*, 67–79. https://doi.org/10.1016/j.semcdb.2018.05.019

Hallgrímsson, B., Willmore, K., & Hall, B. K. (2002). Canalization, developmental stability, and morphological integration in primate limbs. *Yearbook of Physical Anthropology, 45*(S35), 131–158. https://doi.org/10.1002/ajpa.10182

Hendry, A. P. (2009). Ecological speciation! Or the lack thereof? *Canadian Journal of Fisheries and Aquatic Sciences, 66*(8), 1383–1398. https://doi.org/10.1139/F09-074

Hendry, A. P. (2016). Introduction and conceptual framework. In *Eco-Evolutionary Dynamics* (pp. 1–23). https://doi.org/papers3://publication/uuid/74061AE9-0331-4CF3-998D-E4C13D9BCF7A

Hendry, A. P., Berg, O. K., & Quinn, T. P. (2001). Breeding location choice in salmon: Causes (habitat, competition, body size, energy stores) and consequences (life span, energy stores). *Oikos, 93*(3), 407–418. https://doi.org/10.1034/j.1600-0706.2001.930306.x

Holtmann, B., Santos, E. S. A., Lara, C. E., & Nakagawa, S. (2017). Personality-matching habitat choice, rather than behavioural plasticity, is a likely driver of a phenotype–environment covariance. *Proceedings of the Royal Society B: Biological Sciences, 284*(1864), 20170943. https://doi.org/10.1098/rspb.2017.0943

Ingley, S. J., & Johnson, J. B. (2014). Animal personality as a driver of reproductive isolation. *Trends in Ecology and Evolution, 29*(7), 369–371. https://doi.org/10.1016/j.tree.2014.04.008

Jónasson, P. M. (1992). The Ecosystem of Thingvallavatn : A Synthesis. *Oikos, 64*(1), 405–434.

Jones, A. G., Arnold, S. J., & Bürger, R. (2003). Stability of the G-matrix in a population experiencing pleiotropic mutation, stabilizing selection, and genetic drift. *Evolution, 57*, 1747–1760.

Joris, P., Dellinger, M., & Benhaïm, D. (2022). Among-individual variation of risk-taking behaviour in group and solitary context is uncorrelated but independently repeatable in a juvenile Arctic charr (*Salvelinus alpinus*) aquaculture strain. *Applied Animal Behaviour Science*, 105596. https://doi.org/10.1016/j.applanim.2022.105596

Kapralova, K. H., Jónsson, Z. O., Palsson, A., Franzdóttir, S. R., le Deuff, S., Kristjánsson, B. K., & Snorrason, S. S. (2015). Bones in motion: Ontogeny of craniofacial development in sympatric Arctic charr morphs. *Developmental Dynamics, 244*(9), 1168–1178. https://doi.org/10.1002/dvdy.24302

Kapralova, K. H., Morrissey, M. B., Kristjánsson, B. K., Olafsdóttir, G. Á., Snorrason, S. S., & Ferguson, M. M. (2011). Evolution of adaptive diversity and genetic connectivity in

Arctic charr (*Salvelinus alpinus*) in Iceland. *Heredity, 106*(3), 472–487. https://doi.org/10.1038/hdy.2010.161

Kapralova, K.H. (2014). *Study of morphogenesis and miRNA expression associated with craniofacial diversity in Arctic charr (Salvelinus alpinus) morphs*. University of Iceland. Retrieved from http://skemman.is/item/view/1946/19398

Kapralova, Kalina H., Franzdóttir, S. R., Jónsson, H., Snorrason, S. S., & Jónsson, Z. O. (2014). Patterns of MiRNA expression in Arctic charr development. *PLoS ONE, 9*(8). https://doi.org/10.1371/journal.pone.0106084

Kapralova, Kalina H., Guðbrandsson, J., Reynisdottir, S., Santos, C. B., Baltanás, V. C., Maier, V. H., … Palsson, A. (2013). Differentiation at the MHCIIα and Cath2 Loci in sympatric *Salvelinus alpinus* resource morphs in Lake Thingvallavatn. *PLoS ONE, 8*(7). https://doi.org/10.1371/journal.pone.0069402

Kern, E. M. A., Robinson, D., Gass, E., Godwin, J., & Langerhans, R. B. (2016). Correlated evolution of personality, morphology and performance. *Animal Behaviour, 117*, 79–86. https://doi.org/10.1016/j.anbehav.2016.04.007

Kjartansson, G. (1964). Aldur nokkurra hrauna á Suðulandi. *Náttúrufræðingurinn, 34*(3), 101–126.

Kristjánsson, B. K., Skúlason, S., & Noakes, D. L. G. (2002). Morphological segregation of Icelandic threespine stickleback (*Gasterosteus aculeatus L*). *Biological Journal of the Linnean Society, 76*(2), 247–257. https://doi.org/10.1046/j.1095-8312.2002.00063.x

Krzanowski, W. J. (1979). Between-Groups Comparison of Principal Components. Journal of the American Statistical Association, 74(367), 703–707.

Kulmuni, J., Butlin, R. K., Lucek, K., Savolainen, V., & Westram, A. M. (2020). Towards the completion of speciation: the evolution of reproductive isolation beyond the first barriers. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 375*(1806), 20190528. https://doi.org/10.1098/rstb.2019.0528

Küttner, E., Parsons, K. J., Easton, A. A., Skúlason, S., Danzmann, R. G., & Ferguson, M. M. (2014). Hidden genetic variation evolves with ecological specialization: the genetic basis of phenotypic plasticity in Arctic charr ecomorphs. *Evolution & Development*, *16*(4), 247–257. https://doi.org/10.1111/ede.12087

Küttner, E., Parsons, K. J., Robinson, B. W., Skúlason, S., Danzmann, R. G., & Ferguson, M. M. (2013). Effects of population, family, and diet on craniofacial morphology of Icelandic Arctic charr (*Salvelinus alpinus*). *Biological Journal of the Linnean Society, 108*(3), 702–714. https://doi.org/10.1111/j.1095-8312.2012.02038.x

Lackey, A. C. R., & Boughman, J. W. (2017). Evolution of reproductive isolation in stickleback fish. *Evolution, 71*(2), 357–372. https://doi.org/10.1111/evo.13114

Laland, K., Uller, T., Feldman, M., Sterelny, K., Müller, G. B., Moczek, A., … Strassmann, J. E. (2014). Does evolutionary theory need a rethink? *Nature, 514*(7521), 161–164. https://doi.org/10.1038/514161a

Lazić, M. M., Carretero, M. A., Crnobrnja-Isailović, J., & Kaliontzopoulou, A. (2014). Effects of environmental disturbance on phenotypic variation: An Integrated assessment of canalization, developmental stability, modularity, and allometry in lizard head shape. The American Naturalist, 185(1), 44–58. https://doi.org/10.1086/679011

Leblanc, C. A. L., Benhaïm, D., Hansen, B. R., Kristjánsson, B. K., & Skúlason, S. (2011). The importance of egg size and social effects for behaviour of Arctic charr juveniles. *Ethology, 117*(8), 664–674. https://doi.org/10.1111/j.1439-0310.2011.01920.x

Mack, K. L., Campbell, P., & Nachman, M. W. (2016). Gene regulation and speciation in house mice. Genome Research, 26, 451–465. https://doi.org/10.1016/j.tig.2016.11.003

Magnússon, K. G. . (1992). Birds of the Thingvallavatn area. Oikos, 64(1), 381–395.

Magnusson, K. P., & Ferguson, M. M. (1987). Genetic analysis of four sympatric morphs of Arctic charr, *Salvelinus alpinus*, from Thingvallavatn, Iceland. *Environmental Biology of Fishes, 20*(1), 67–73. https://doi.org/10.1007/BF00002026

Malmquist, A. H. J., Snorrason, S. S., Skúlason, S., Jonsson, B., Sandlund, O. T., & Jonasson, M. (1992). Diet differentiation in polymorphic Arctic charr in Thingvallavatn, Iceland. *Journal of Animal Ecology, 61*(1), 21–35.

Malmquist, H. J. (1992). Phenotype-specific feeding behaviour of two Arctic charr *Salvelinus alpinus* morphs. *Oecologia, 92*(3), 354–361.

Malmquist, Hilmar J., Antonsson, T., Gudbergssion, G., Skúlason, S., & Snorrason, S. S. (2000). Biodiversity of macroinvertebrates on rocky substrate in the surf zone of Icelandic lakes. *Verhandlungen Des Internationalen Verein Limnologie*, *27*(1), 121–127.

Malmquist, Hilmar J., Snorrason, S. S., & Skúlason, S. (1985). Bleikjan í Þingvallavatni. I. Fæðuhættir. *Náttúrufræðingurinn, 55*(4), 195–217.

Marklund, M. H. K., Svanbäck, R., Faulks, L., Breed, M. F., Scharnweber, K., Zha, Y., & Eklöv, P. (2019). Asymmetrical habitat coupling of an aquatic predator — The importance of individual specialization. *Ecology and Evolution, 1*(11), 1–11. https://doi.org/10.1002/ece3.4973

Matute, D. R., & Cooper, B. S. (2021). Comparative studies on speciation: 30 years since Coyne and Orr. *Evolution, 75*(4), 764–778. https://doi.org/10.1111/evo.14181

McGirr, J. A., & Martin, C. H. (2019). Hybrid gene misregulation in multiple developing tissues within a recent adaptive radiation of *Cyprinodon* pupfishes. *PLOS ONE, 14*(7), e0218899. https://doi.org/10.1371/journal.pone.0218899

Metcalfe, N. B., & Monaghan, P. (2001). Compensation for a bad start: grow now, pay later? *Trends in Ecology & Evolution, 16*(5), 254–260. https://doi.org/10.1016/S0169-5347(01)02124-3

Nicieza, A., & Metcalfe, N. B. (1997). Growth compensation in juvenile atlantic salmon : responses to depressed temperature and food availability. *Ecology, 78*(8), 2385–2400.

Nosil, P. (2012a). *Ecological Speciation* (1st ed.). Oxford: Oxford University Press.

Nosil, P. (2012b). The speciation continuum: what factors affect how far speciation proceeds? In *Ecological Speciation* (p. 208).

Nosil, P., Harmon, L. J., & Seehausen, O. (2009). Ecological explanations for (incomplete) speciation. *Trends in Ecology and Evolution, 24*(3), 145–156. https://doi.org/10.1016/j.tree.2008.10.011

Ólafsdóttir, G. Á., Ritchie, M. G., & Snorrason, S. S. (2006). Positive assortative mating between recently described sympatric morphs of Icelandic sticklebacks. *Biology Letters, 2*(2), 250–252. https://doi.org/10.1098/rsbl.2006.0456

Parsons, K. J., Sheets, H. D., Skúlason, S., & Ferguson, M. M. (2011). Phenotypic plasticity, heterochrony and ontogenetic repatterning during juvenile development of divergent arctic charr (*Salvelinus alpinus). Journal of Evolutionary Biology, 24*(8), 1640–1652. https://doi.org/10.1111/j.1420-9101.2011.02301.x

Parsons, K. J., Skúlason, S., & Ferguson, M. (2010). Morphological variation over ontogeny and environments in resource polymorphic arctic charr (*Salvelinus alpinus*). *Evolution and Development, 12*(3), 246–257. https://doi.org/10.1111/j.1525-142X.2010.00410.x

Parsons, K. J., Son, Y. H., & Craig Albertson, R. (2011). Hybridization promotes evolvability in African cichlids: Connections between transgressive segregation and phenotypic integration. *Evolutionary Biology, 38*(3), 306–315. https://doi.org/10.1007/s11692-011-9126-7

Parsons, K. J., Trent Taylor, A., Powder, K. E., & Albertson, R. C. (2014). Wnt signalling underlies the evolution of new phenotypes and craniofacial variability in Lake Malawi cichlids. *Nature Communications*, *5*, 1–11. https://doi.org/10.1038/ncomms4629

Pavey, S. A., Collin, H., Nosil, P., & Rogers, S. M. (2010). The role of gene expression in ecological speciation. *Annals of the New York Academy of Sciences, 1206*, 110–129. https://doi.org/10.1111/j.1749-6632.2010.05765.x

Pélabon, C., Carlson, M. L., Hansen, T. F., Yoccoz, N. G., & Armbruster, W. S. (2004). Consequences of inter-population crosses on developmental stability and canalization of floral traits in *Dalechampia scandens* (Euphorbiaceae). *Journal of Evolutionary Biology, 17*(1), 19–32. https://doi.org/10.1046/j.1420-9101.2003.00663.x

Pesevski, M., & Dworkin, I. (2020). Genetic and environmental canalization are not associated among altitudinally varying populations of *Drosophila melanogaster*. *Evolution, 74*(8), 1755–1771. https://doi.org/10.1111/evo.14039

Pétursson, H. G., Norðdahl, H., & Ingólfsson. (2015). Late Weichselian history of relative sea level changes in Iceland during a collapse and subsequent retreat of marine based ice sheet. *Cuadernos de Investigación Geográfica, 41*(2), 261–277. https://doi.org/10.18172/cig.2741

Pfennig, D. W., Wund, M. A., Snell-rood, E. C., Cruickshank, T., Schlichting, C. D., & Moczek, A. P. (2010). Phenotypic plasticity' s impacts on diversification and

speciation. *Trends in Ecology & Evolution, 25*(8), 459–467. https://doi.org/10.1016/j.tree.2010.05.006

Polverino, G., Santostefano, F., Díaz-Gil, C., & Mehner, T. (2018). Ecological conditions drive pace-of-life syndromes by shaping relationships between life history, physiology and behaviour in two populations of Eastern mosquitofish. *Scientific Reports, 8*(1), 1–10. https://doi.org/10.1038/s41598-018-33047-0

Ponsioen, L. (2020). *Reproductive barriers between sympatric morphs of Arctic charr (Salvelinus alpinus) in lake Thingvallavatn, Iceland*. University of Iceland.

Quinn, T. P. (2018). *The behavior and ecology of Pacific Salmon and Trout.* (U. of W. Press, Ed.) (Second edi).

Rabosky, D. L., & Matute, D. R. (2013). Macroevolutionary speciation rates are decoupled from the evolution of intrinsic reproductive isolation in *Drosophila* and birds. *Proceedings of the National Academy of Sciences of the United States of America, 110*(38), 15354–15359. https://doi.org/10.1073/pnas.1305529110

Ramsey, J., Bradshaw, H. D., & Schemske, D. W. (2003). Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution, 57*(7), 1520–1534. https://doi.org/10.2307/3448754

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews, 82*(2), 291–318. https://doi.org/10.1111/j.1469-185X.2007.00010.x

Renaut, S., Nolte, A. W., & Bernatchez, L. (2009). Gene expression divergence and hybrid misexpression between lake whitefish species pairs (*Coregonus spp*. Salmonidae). *Molecular Biology and Evolution, 26*(4), 925–936. https://doi.org/10.1093/molbev/msp017

Rice, A. M., & McQuillan, M. A. (2018). Maladaptive learning and memory in hybrids as a reproductive isolating barrier. *Proceedings of the Royal Society B: Biological Sciences, 285*(1879), 20180542. https://doi.org/10.1098/rspb.2018.0542

Roche, D. G., Careau, V., & Binning, S. A. (2016). Demystifying animal 'personality' (or not): why individual variation matters to experimental biologists. *Journal of Experimental Biology, 219*(24), 3832–3843. https://doi.org/10.1242/jeb.146712

Rohlf, F. J., & Corti, M. (2000). Use of two block partial least squares to study covariation in shape. *Syst. Biol., 49*(4), 740–753.

Rolan-Alvarez, E., & Caballero, A. (2000). Estimating sexual selection and sexual isolation effects from mating frequencies. *Evolution, 54*(1), 30–36.

Royauté, R., Hedrick, A., & Dochtermann, N. A. (2020). Behavioral syndromes shape evolutionary trajectories via conserved genetic architecture. *Proceedings of the Royal Society B: Biological Sciences, 287*(1927), 20200183. https://doi.org/10.1101/619411

Sæmundsson, K. (1992). Geology of Thinvallavatn area. *Oikos, 64*, 40–68.

Sandlund, O. T., Gunnarsson, K., Jónasson, P. M., Jonsson, B., Lindem, T., Magnússon, K. P., … Snorrason, S. S. (1992). The Arctic charr *Salvelinus alpinus* in Thingvallavatn. *Oikos, 64*(12), 305–351. https://doi.org/https://doi.org/10.2307/3545056

Sandlund, O. T., Jonsson, B., Malmquist, H. J., Gydemo, R., Lindem, T., Skúlason, S., … Jónasson, P. M. (1987). Habitat use of arctic charr *Salvelinus alpinus* in Thinvgallavatn, Iceland. *Environmental Biology of Fishes, 20*(4), 263–264.

Sandlund, O. T., Malmquist, H. J., Jonsson, B., Skúlason, S., Snorrason, S. S., Jónasson, P. M., … Lindem, T. (1988). Density, length distribution, and diet of age-0 Arctic charr *Salvelinus alpinus* in the surf zone of Thingvallavatn, Iceland. *Environmental Biology of Fishes, 23*(3), 183–195. https://doi.org/10.1007/BF00004909

Schluter, D. (2009). Evidence for ecological speciation and its alternative. *Science, 323*(5915)*,* 737–741. https://doi.org/10.1126/science.1160006

Schluter, Dolph. (1996). Ecological speciation in postglacial fishes. *Philosophical Transactions of the Royal Society B: Biological Sciences, 351*(1341), 807–814. https://doi.org/10.1098/rstb.1996.0075

Seehausen, O. (2004). Hybridization and adaptive radiation. *Trends in Ecology and Evolution, 19*(4), 198–207. https://doi.org/10.1016/j.tree.2004.01.003

Seehausen, O., Butlin, R. K., Keller, I., Wagner, C. E., Boughman, J. W., Hohenlohe, P. A., … Widmer, A. (2014). Genomics and the origin of species. *Nature Reviews Genetics, 15*(3), 176–192. https://doi.org/10.1038/nrg3644

Selz, O. M., Lucek, K., Young, K. A., & Seehausen, O. (2014). Relaxed trait covariance in interspecific cichlid hybrids predicts morphological diversity in adaptive radiations. *Journal of Evolutionary Biology, 27*(1), 11–24. https://doi.org/10.1111/jeb.12283

Sigurjónsdóttir, H., & Gunnarsson, A. (1989). Alternative mating tactics of arctic charr, *Salvelinus alpinus*, in Thingvallavatn, Iceland. *Environmental Biology of Fishes, 26*(3), 159–176. https://doi.org/10.1007/BF00004814

Sih, A., Cote, J., Evans, M., Fogarty, S., & Pruitt, J. (2012). Ecological implications of behavioural syndromes. *Ecology Letters, 15*(3), 278–289. https://doi.org/10.1111/j.1461-0248.2011.01731.x

Skúlason, S, Snorrason, S. S., Noakes, D. L., & Ferguson, M. M. (1996). Genetic basis of life history variations among sympatric morphs of Arctic char (*Salvelinus alpinus*). *Canadian Journal of Fisheries and Aquatic Sciences, 53*(8), 1807–1813. https://doi.org/10.1139/f96-098

Skúlason, Skúli, Noakes, D. L. G., & Snorrason, S. S. (1989). Ontogeny of trophic morphology in four sympatric morphs of Arctic charr *Salvelinus alpinus* in Thingvallavatn, Iceland. *Biological Journal of the Linnean Society, 38*(3), 281–301. https://doi.org/10.1111/j.1095-8312.1989.tb01579.x

Skúlason, Skúli, Parsons, K. J., Svanbäck, R., Räsänen, K., Ferguson, M. M., Adams, C. E., … Snorrason, S. S. (2019). A way forward with eco evo devo: an extended theory of

resource polymorphism with postglacial fishes as model systems. *Biological Reviews, 94*(5), 1786–1808. https://doi.org/10.1111/brv.12534

Skúlason, Skúli, & Smith, T. B. (1995). Resource polymorphisms in vertebrates. *Trends in Ecology & Evolution, 10*(9), 366–370. https://doi.org/10.1016/S0169-5347(00)89135-1

Skúlason, Skúli, Snorrason, S. S., Noakes, D. L. G., Ferguson, M. M., & Malmquist, H. J. (1989). Segregation in spawning and early life history among polymorphic Arctic charr, *Salvelinus alpinus*, in Thingvallavatn, Iceland. *Journal of Fish Biology, 35*, 225–232. https://doi.org/10.1111/j.1095-8649.1989.tb03065.x

Skúlason, Skúli, Snorrason, S. S., Ota, D., & Noakes, D. L. G. (1993). Genetically based differences in foraging behaviour among sympatric morphs of Arctic charr (Pisces: Salmonidae). *Animal Behaviour, 45*(6), 1179–1192. https://doi.org/https://doi.org/10.1006/anbe.1993.1140

Smith, S. R., Normandeau, E., Djambazian, H., Nawarathna, P. M., Berube, P., Muir, A. M., … Bernatchez, L. (2022). A chromosome-anchored genome assembly for Lake Trout (*Salvelinus namaycush*). *Molecular Ecology Resources, 22*(2), 679–694. https://doi.org/10.1111/1755-0998.13483

Smith, T. B., & Skúlason, S. (1996). Evolutionary significance of resource polymorphisms in fishes , amphibians , and birds. *Annual Review of Ecology and Systematics, 27*(1), 111–133.

Snorrason. (2000). Life cycle of the mollusc *Lymnaea peregra* and its influence on morph formation in Arctic charr, *Salvelinus alpinus*. *Verhandlungen Des Internationalen Verein Limnologie, 27*, 1–4.

Snorrason, S.S., Skúlason, S., Sandlund, O. T., Malmquist, H. J., Jonsson, B., & Jónasson, P. M. (1989). Shape polymorphism in Arctic charr, *Salvelinus alpinus*, in Thingvallavatn, Iceland. *Physiolg Ecology Japan, 1*, 393–404.

Snorrason, Sigurður S., Jónasson, P. M., Jonsson, B., Lindem, T., Malmquist, H. J., Sandlund, O. T., & Skúlason, S. (1992). Population dynamics of the planktivorous arctic charr *Salvelinus alpinus* (murta') in Thingvallavatn. *Oikos, 64*, 352–364.

Snorrason, Sigurður Sveinn, Skúlason, S., Jonsson, B., Malmquist, H. J., Jónasson, P. M., Sandlund, O. T., & Lindem, T. (1994). Trophic specialization in Arctic charr *Salvelinus alpinus* (Pisces; Salmonidae): morphological divergence and ontogenetic niche shifts. *Biological Journal of the Linnean Society, 52*(1), 1–18. https://doi.org/10.1111/j.1095-8312.1994.tb00975.x

Sobel, J. M., & Chen, G. F. (2014). Unification of methods for estimating the strength of reproductive isolation. *Evolution, 68*(5), 1511–1522. https://doi.org/10.1111/evo.12362

Sobel, J. M., Chen, G. F., Watt, L. R., & Schemske, D. W. (2010). *The biology of speciation. Evolution, 64*(2), 295–315. https://doi.org/10.1111/j.1558-5646.2009.00877.x

Stankowski, S., & Ravinet, M. (2021). Defining the speciation continuum. *Evolution, 75*(6), 1256–1273. https://doi.org/10.1111/evo.14215

Stelkens, R. B., Schmid, C., Selz, O., & Seehausen, O. (2009). Phenotypic novelty in experimental hybrids is predicted by the genetic distance between species of cichlid fish. *BMC Evolutionary Biology, 9*(1), 283. https://doi.org/10.1186/1471-2148-9-283

Svensson, E. I. (2021). The structure of evolutionary theory : Beyond Neo-Darwinism , Neo-Lamarckism and biased historical narratives about the Modern Synthesis, 1–84.

Thompson, K. A., Peichel, C. L., Rennison, D. J., McGee, M. D., Albert, A. Y. K., Vines, T. H., … Schluter, D. (2022). Analysis of ancestry heterozygosity suggests that hybrid incompatibilities in threespine stickleback are environment dependent. *PLOS Biology, 20*(1), e3001469. https://doi.org/10.1371/journal.pbio.3001469

Thompson, K. A., Urquhart-Cronish, M., Whitney, K. D., Rieseberg, L. H., & Schluter, D. (2021). Patterns, Predictors, and Consequences of Dominance in Hybrids. *The American Naturalist, 197*(3), E72–E88. https://doi.org/10.1086/712603

Tigano, A., & Friesen, V. L. (2016). Genomics of local adaptation with gene flow. *Molecular Ecology, 25*(10), 2144–2164. https://doi.org/10.1111/mec.13606

Uller, T., Moczek, A. P., Watson, R. A., Brakefield, P. M., & Laland, K. N. (2018). Developmental bias and evolution: A regulatory network perspective. *Genetics, 209*(4), 949–966. https://doi.org/10.1534/genetics.118.300995

Volpe, J. P., & Ferguson, M. M. (1996). Molecular genetic examination of the polymorphic Arctic charr *Salvelinus alpinus* of Thingvallavatn, Iceland. *Molecular Ecology, 5*(6), 763–772. https://doi.org/10.1111/j.1365-294X.1996.tb00372.x

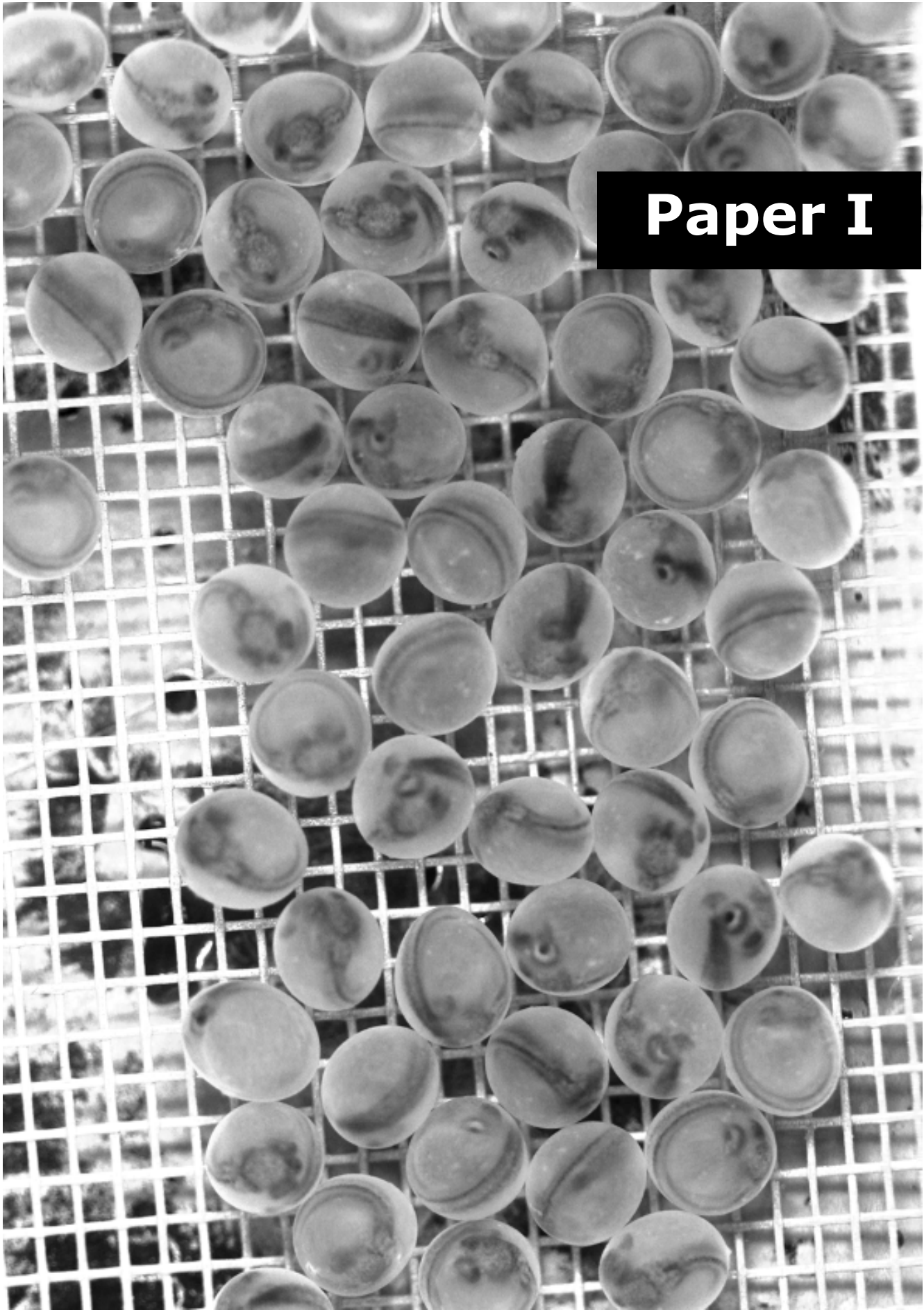
Wagner, G. P., Booth, G., & Bagheri-Chaichian, H. (1997). A population genetic theory of canalization. *Evolution, 51*(2), 329–347. https://doi.org/10.1111/j.1558-5646.1997.tb02420.x

Wilson, A. J., Gíslason, D., Skúlason, S., Snorrason, S. S., Adams, C. E., Alexander, G., … Ferguson, M. M. (2004). Population genetic structure of Arctic Charr, *Salvelinus alpinus* from northwest Europe on large and small spatial scales. *Molecular Ecology, 13*(5), 1129–1142. https://doi.org/10.1111/j.1365-294X.2004.02149.x

Wilson, D. S. (1998). Adaptive individual differences within single populations. *Philosophical Transactions of the Royal Society B: Biological Sciences, 353*(1366), 199–205. https://doi.org/10.1098/rstb.1998.0202

Wimberger, P. H. (1992). Plasticity of fish body shape. The effects of diet, development, family and age in two species of *Goephagus* (Pisces: Cichlidae). *Biological Journal of the Linnean Society*, 45, 197–218.

Wolf, M., & Weissing, F. J. (2012). Animal personalities: Consequences for ecology and evolution. *Trends in Ecology and Evolution, 27*(8), 452–461. https://doi.org/10.1016/j.tree.2012.05.001


Paper I

BMC Ecology and Evolution

RESEARCH

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Multivariate analysis of morphology, behaviour, growth and developmental timing in hybrids brings new insights into the divergence of sympatric Arctic charr morphs

Quentin J.-B. Horta-Lacueva[1*], Sigurður S. Snorrason[1], Michael B. Morrissey[2], Camille A.-L. Leblanc[3] and Kalina H. Kapralova[1]

Abstract

Background: Studying the development of fitness related traits in hybrids from populations diverging in sympatry is a fundamental approach to understand the processes of speciation. However, such traits are often affected by covariance structures that complicate the comprehension of these processes, especially because the interactive relationships between traits of different nature (e.g. morphology, behaviour, life-history) remain largely unknown in this context. In a common garden setup, we conducted an extensive examination of a large suit of traits putatively involved in the divergence of two morphs of Arctic charr (*Salvelinus alpinus*), and investigated the consequences of potential patterns of trait covariance on the phenotype of their hybrids. These traits were measured along ontogeny and involved growth, yolk sac resorption, developmental timing (hatching and the onset of exogeneous feeding), head morphology and feeding behaviour.

Results: Growth trajectories provided the strongest signal of phenotypic divergence between the two charr. Strikingly, the first-generation hybrids did not show intermediate nor delayed growth but were similar to the smallest morph, suggesting parental biases in the inheritance of growth patterns. However, we did not observe extensive multivariate trait differences between the two morphs and their hybrids. Growth was linked to head morphology (suggesting that morphological variations in early juveniles relate to simple allometric effects) but this was the only strong signal of covariance observed between all the measured traits. Furthermore, we did not report evidence for differences in overall phenotypic variance between morphs, nor for enhanced phenotypic variability in their hybrids.

Conclusion: Our study shed light on the multivariate aspect of development in a context of adaptive divergence. The lack of evidence for the integration of most traits into a single covariance structure suggested that phenotypic constraints may not always favour nor impede divergence toward ecological niches differing in numerous physical and ecological variables, as observed in the respective habitats of the two charr. Likewise, the role of hybridization as

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a disruptive agent of trait covariance may not necessarily be significant in the evolution of populations undergoing resource polymorphism.

Keywords: Adaptive divergence, Ecological speciation, Development, Trait covariance, Sympatry, Resource polymorphism, Hybridization

Background

Understanding how phenotypic traits subjected to divergent selection evolve is essential to comprehend the processes of adaptive divergence and speciation [1–4]. In this context, reproductive isolation often relates to reduced fitness in hybrids whose values for specific traits under divergent selection are intermediate or fall outside of the range of parental values (i.e. transgressive characters) [5–7]. However, traits are rarely independent entities because of functional trade-offs [8, 9], developmental constraints [10], genetic constraints like pleiotropy and linkage disequilibrium [10, 11] or the effect of correlational selection [12, 13]. Furthermore, traits belonging to various processes (i.e. life-history, development, behaviour) and encompassing different ontogenetic stages are often intertwined (for examples in fish and amphibians, see [14–23]. While these evolutionary aspects have long been studied in the field of quantitative genetics, and while classical models of ecological speciation are based on the effects of pleiotropy and/or of large sets of co-selected genes [2, 24, 25], little is known about the importance of covarying traits in a context of speciation [2, 6], especially regarding the development of the hybrid phenotypes. Studies on hybridisation often focus on one or a limited number of traits, most often related to morphology and to some extent to physiology and behaviour [2] (but see [26], for a thorough study on life-history and morphology), which reveals the need for multivariate, longitudinal studies on the ontogeny of hybrids.

Characterizing the development of first-generation hybrids (F_1 hybrids) in a multivariate framework would be a first significant step to understand the effects of trait covariance in speciation. Additive mechanisms generating intermediate mean trait values in F_1 hybrids are expected to be fairly common [1, 27–29]. However, recent theoretical and empirical studies report evidence for dominance in individual traits often causing parent bias (i.e. hybrids having closer trait values to one parent rather than being intermediate [30, 31] or showing extreme phenotypes [32–37]). In addition to mean trait values, increased phenotypic variance in F_1 hybrids is expected, presumably because of new allelic combinations and epistatic effects [27]. Likewise, trait covariance and correlations should be strengthened in many cases [27], but hybridization is also expected to relax trait correlations [38]. Finally, independent traits affected by parent-bias are likely to generate "trait mismatches" that might be detrimental in the wild [30]. Given the high number of traits potentially involved in divergence processes and the importance of trait covariance, it becomes critical to thoroughly study the development of F_1 hybrids in a multivariate context before studying the evolutionary consequences (e.g. selection against hybrids as a reproductive barrier).

Polymorphic fish from Northern freshwater lakes are particularly well-suited models to study the processes of phenotypic divergence [39]. The evolution of these fish fits the narrative of resource polymorphism, through which different forms (i.e. morphs) have emerged from ancestral populations that invaded multiple, unoccupied niches within the same geographical system [40]. Such diversification often follows the colonisation of deglaciated lakes, where the diverging morphs (generally segregating between benthic and pelagic habitats) differ in morphology, life-history traits and/or behaviour [41, 42]. Various levels of reproductive isolation are encountered among these systems, ranging from single populations with continuous variation, to discrete varieties with more-or-less reversible reproductive barriers, to completely reproductively isolated species [5, 43, 44]. In recent years a growing number of cases have been reported where post-glacial morphs are found (at least in their current state) in sympatry [44–46]. These geographical and evolutionary systems facilitate the explorations of the mechanisms of adaptive divergence and speciation because of the reduced confounding effects of long and complex evolutionary histories [47].

Using multivariate phenotypic data on morphology, behaviour and ontogeny, and considering different developmental stages, we characterized phenotypic variations among two of the four sympatric morphs of Arctic charr (*Salvelinus alpinus*) from Thingvallavatn, Iceland, and of their hybrids. These morphs are the small-benthic (SB) and the planktivorous charr (PL), which constitute two genetically differentiated populations [48–50] and differ in head and body shape, habitat use, diet, life-history and parasites [51–53]. The SB charr live in the interstitial spaces of a lava matrix forming the stony littoral zone of the lake, where they forage on benthic invertebrates. The PL charr utilize the pelagic zone of the lake where they feed on zooplankton and emerging chironomids. Because these two habitats differ extensively in their physical and

ecological characteristics [53, 54], the different selective regimes experienced by each morph are expected to affect a wide variety of traits. Previous studies already indicate that the PL and the SB charr have evolved genetically based differences in their embryonic growth [52], craniofacial development [55, 56], and foraging strategy [57]. The two morphs overlap in their spawning time and places [58] but recent estimates of gene flow indicate substantial reproductive isolation [49, 50]. Fertile hybrids (at least of the generation F_1) can however easily be produced in laboratory. In the wild, selection against hybrids is therefore likely to be an important reproductive barrier between these two morphs.

Using a common garden set-up, we reared the offspring of SB, PL charr and their hybrids, keeping track of individuals from hatching until about 3 months after the onset of exogeneous feeding. We assessed traits related to morphology and development (hatching date, initial size and growth, yolk sac size and resorption, developmental trajectory of the head shape). These measurements enabled us not only to test for differences in average value, variances and covariances of traits between types of crosses, but also to assess whether and how these traits covary with other traits measured later in life, and which were related to morphology (shape of the feeding apparatus), behaviour (feeding intensity) and growth after the onset of exogeneous feeding (Table 1). We first hypothesised that the two morphs have rapidly diverged in every aspect of their developmental phenotype. If the two morphs have evolved towards distinct multivariate fitness optima, we expected to observe (1) differences between pure-morph offspring in average trait values. Because divergence may affect already covarying traits or involve correlational selection, we also expected (2) differences in trait variances and covariances to be established between the two pure-morph offspring.

Our second hypothesis was that hybrids show a unique ontogenetic phenotype composed of characters with various inheritance patterns (additive, dominant, over dominant). These characters would provide cues regarding the potential of reproductive isolation and/or hybridization to generate phenotypic variation.

Table 1 Variables selected for generating the phenotypic (**P**) variance–covariance matrices (one per cross type)

Category	Variable name
Development	Standard length at hatching (D1)
	Standard length at the onset of first feeding (D3)
	Growth from hatching (D1) to 20 days post-hatching (D2)
	Growth from 3–4 weeks after the onset of exogeneous feeding (D3) to 9–11 weeks after the onset of exogeneous feeding (D4)
	Yolk sac size at hatching (D1)
	Yolk sac conversion
Behaviour	Latency to start feeding at the start of observational trials

Results

Developmental deficiencies

We first investigated whether higher mortality or higher occurrence of heavy malformations in hybrids can be observed in our common garden study. The proportion of individuals dying after hatching or killed because of heavy malformations appeared to be higher in the SB × SB offspring and the hybrids than in the PL × PL offspring (PL × PL: 0.03; SB × SB: 0.32; hybrids: 0.29). However, after implementing a Generalized Mixed models (GLMM) with family (i.e. the egg clutch) as a random effect, these differences only appear as trends (posterior modes [95% CrIs] of the survival probability on the latent scale = PL × PL: 3.24 [1.80; 5.83], SB × SB: 0.62 [−0.60; 1.92], hybrids: 0.91 [−0.05; 1.80], family effect: 0.02 [0.00; 2.42]).

Differences at the level of individual traits

We collected multivariate longitudinal individual-based data on ontogeny (standard length, yolk sac resorption, growth before and after the onset of exogeneous feeding, timing of the onset of exogeneous feeding), trophic morphology (head shape) and feeding behaviour (feeding activity and feeding performance). With the exception of shape data, differences in average trait value and in variances were estimated by fitting GLMMs and by making inferences based on the overlap between 95% High Posterior Credible intervals (95% CrI).

Longitudinal size measurements (standard length) indicated that the SB × SB and the hybrids differed from the PL × PL offspring in their growth trajectories (Fig. 1). We observed a trend for lower intercepts in the SB × SB offspring and the hybrids than in PL × PL offspring (posterior modes [95% CrIs] of $\log_{10}$(standard length) = PL × PL: 3.09 [2.97; 3.19], SB × SB: 2.98 [2.90; 3.11], hybrids: 2.98 [2.92; 3.07]). Furthermore, lower slopes and small second order polynomial terms were observed in the SB × SB offspring and the hybrids compared to the PL × PL offspring (slopes = PL × PL: 6.14 [5.89; 6.38], SB × SB: 5.77 [5.38, 5.95], hybrids: 5.73 [5.55; 5.92]; second order polynomial terms = PL × PL: −0.70 [−0.85; −0.55], SB × SB = −1.14 [−1.37; −0.99], hybrids: −1.00 [−1.11; −0.85]). These results indicate a slower and a more decelerating growth in the SB × SB offspring and the hybrids than in the PL × PL offspring.

Using Geometric morphometric data from photographs of the embryos, we did not observe strong differences between the types of crosses in mean yolk

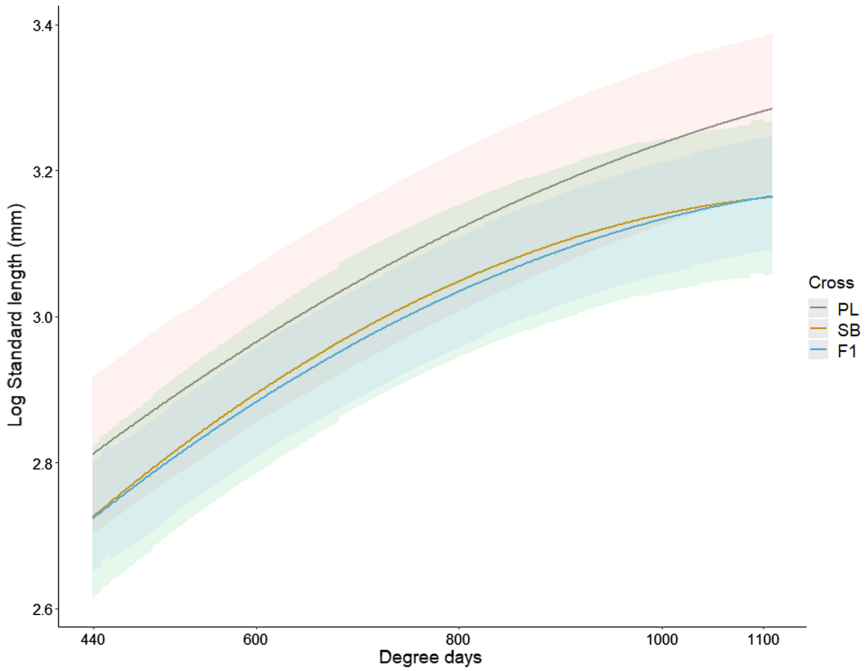

Fig. 1 Growth trajectories of each cross type. The growth period under study started at hatching (ca. 400 °C d) and ended 3 months after the onset of exogeneous feeding (ca. 1100 °C d). PL: PL × PL offspring, SB: SB × SB offspring, F_1: first-generation hybrids. Lines: predicted values, bands: 95% confidence intervals

Table 2 Posterior estimates of the fixed effects from the Multiresponse Generalized Linear Mixed Effect Model on the yolk sac area (mm^2)

	Posterior mode	95% CrI
Response (yolk area at D1)	2.65	1.86; 3.65
Response (yolk area at D2)	2.63	1.56; 3.36
Yolk area at D1 × log(standard length at D1)	0.11	0.06; 0.15
Yolk area at D2 × log(standard length at D2)	0.11	0.06; 0.15
Yolk area at D1 × Cross type SB × SB	− 0.15	− 1.38; 1.17
Yolk area at D2 × Cross type SB × SB	− 0.03	− 1.25; 1.29
Yolk area at D1 × Cross type F_1 hybrids	− 0.21	− 1.21; 0.93
Yolk area at D2 × Cross type F_1 hybrids	− 0.16	− 1.16; 0.99

The PL × PL cross type is the base line. D1: hatching, D2: 20 days post-hatching. See Additional file 1: Table S5 for the details of the model

sac area at hatching nor in the rate of yolk sac resorption (Table 2). The hybrids and the SB × SB offspring appeared to have smaller yolk sac sizes at hatching and the hybrids tended to have faster resorption rate, although wide overlaps in 95% intervals confer low levels of certainty to these patterns.

Head shape variation between cross types was estimated with Analyses of the Procrustes residuals (Randomized Residuals Permutation Procedure) of Geometric Morphometric data from the same set of photographs used for the yolk sac analyses. These analyses indicated that size was related to most of the variation among specimens while no effect of the cross type in itself was observed (Table 3). The ontogenetic trajectories of the head shape did not differ significantly between the types of crosses (Fig. 2, Additional file 1: Table S1). No differences between types of crosses in the variances of the head shapes were observed from the disparity analyses at hatching and at the onset of exogeneous feeding (absolute differences in Procrustes variances < 0001, p-value > 0.1 in all the pairwise comparisons).

Finally, we estimated the date of the onset of exogenous feeding of each individual through daily observations and studied variations in feeding behaviour among cross types (3–4 weeks after the onset of exogeneous feeding) by conducing three sessions of behavioural observations per individual (focal sampling [59]). We did not observe

Table 3 Formula and results of the regression on Procrustes residuals of the head shapes in the specimen reared individually

Formula					
Procrustes coordinates ~ $\log_{10}$(size) + Cross/Family + $\log_{10}$(size) × Cross/Family + Age × Coss/Family					
Table of variance					
Effects	***d.f***	***SS***	R^2	***Z***	***p***
Log(size)	1	5.17	0.62	7.80	< 0.01
Cross type	2	0.06	0.01	− 1.18	0.88
Age	3	0.63	0.07	9.47	< 0.01
Cross type × Family	5	0.25	0.03	8.07	< 0.01
Cross type × log(size)	2	0.02	0.00	− 2.64	1.00
Cross type × Age	6	0.07	0.01	− 3.09	1.00
Cross type × log(size) × Family	5	0.06	0.01	4.08	< 0.01
Cross type × Age × Family	15	0.10	0.01	3.63	< 0.01
Residuals	626	2.06	0.24	–	–
Total	665	8.40	–	–	–

Families are nested within cross type. Age: Sampling time point, Size: Centroid size

differences among cross types either in the mean or in the variances of the date of the onset of exogeneous feeding, the estimated dates being very close to one another (largest posterior mode difference = 5 days, Additional file 1: Table S2). There was no apparent difference between groups in the propensity to start feeding during the experimental trials on feeding behaviour (PL × PL = 0.76 [0.57; 0.87]; SB × SB = 0.70 [0.53; 0.88]; hybrids = 0.65 [0.51; 0.77]; posterior mode [95% CrI], observed scale). However, the PL × PL offspring showed a higher level of consistent individual differences (repeatability) in their propensity to start feeding (R = 0.41 [0.23; 0.53], posterior mode [95% CrI]) than the SB × SB offspring (R = 0.00 [0.00; 0.25]) and the hybrids (R = 0.00 [0.00; 0.27]). The estimated number of captured food items also appeared slightly lower in PL × PL offspring than in the SB × SB conspecifics, although no strong inference can be made in light of the overlapping 95% CrI (Fig. 3a). PL × PL individuals also tended to show lower variance than the SB × SB individuals and the hybrids in the number of

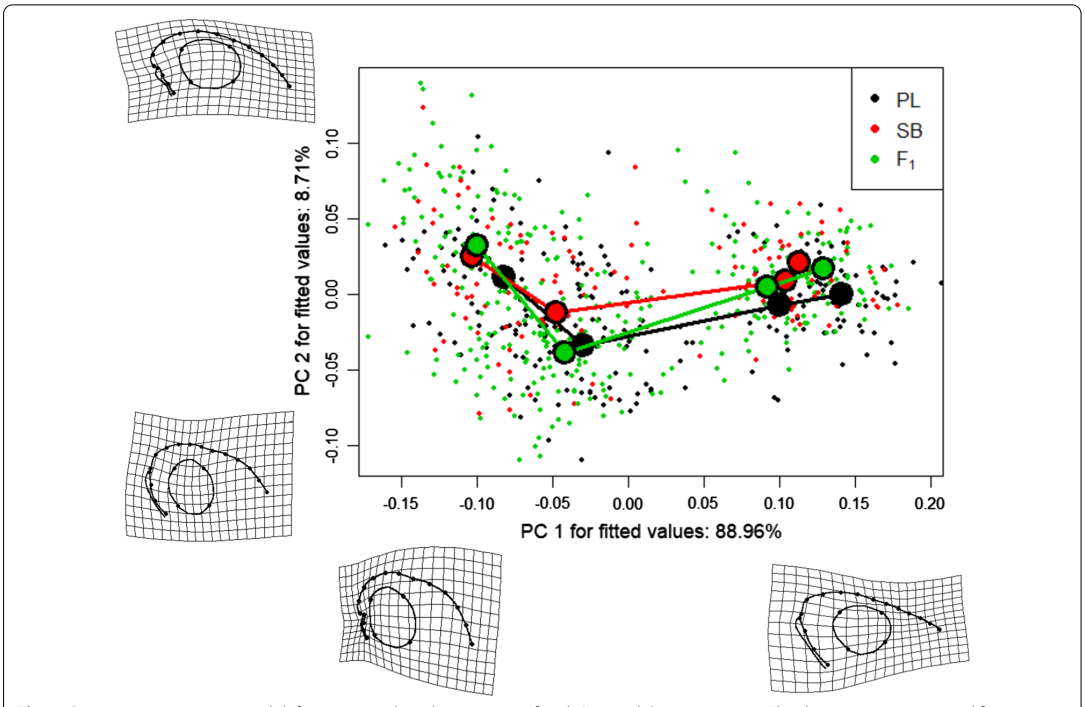

Fig. 2 Ontogenetic trajectories and deformation grids at the extremes of each Principal Component axis. The shapes are not corrected for size. SB = SB × SB offspring, PL = PL × PL offspring, F_1 = firs-generation hybrids. The circles are the mean shapes of each cross type at a given developmental time point (from left to right: hatching, 20 days post hatching, onset of exogeneous feeding, 3 months after the onset of exogeneous feeding)

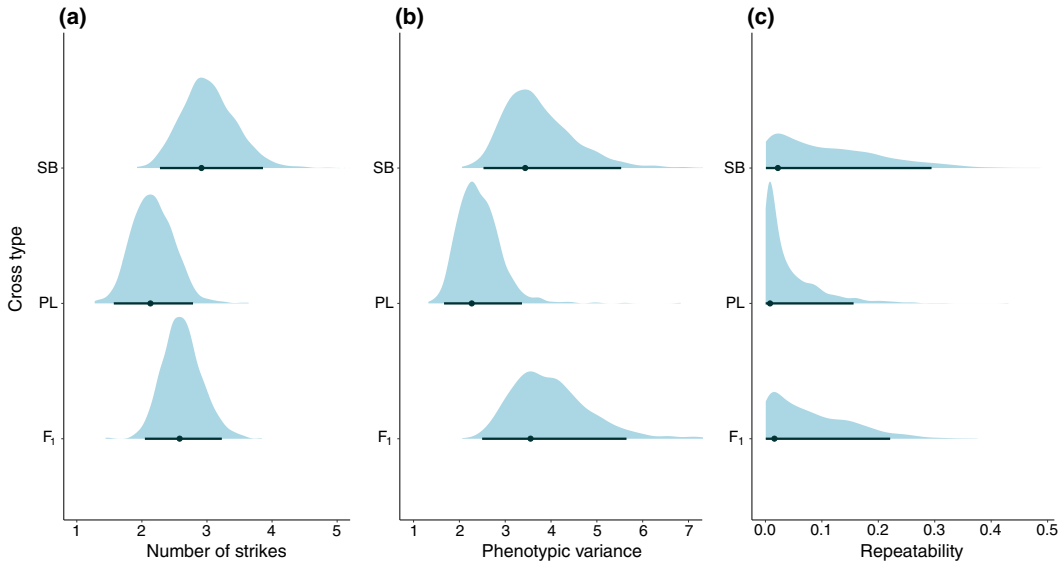

Fig. 3 Number of food items attacked by the focal fish during the feeding trials. **a** Number of attacks, **b** total variance, and **c** Repeatability (R) as a measure of consistent between-individual differences. Categories: SB = SB × SB offspring, PL = PL × PL offspring, F_1 = hybrids. Blue shapes: Posterior densities, dots: Posterior modes, bars: and 95% CrIs

attacked items (Fig. 3b). The comparison of these estimates indicated that the three types of crosses showed low to null levels of consistent differences between individuals in this feeding behaviour (Fig. 3c). We did not observe differences between cross types in the latency to start feeding (all differences in posterior mode > 1 s.; all 95‰ CrI highly similar, Additional file 1: Table S3) nor in the propensity to use the bottom of the container, the water column or the surface of the water when foraging (Additional file 1: Figure S1a–i).

Trait covariance structure and correlations (head shape excluded)

We studied the patterns of trait covariance (excluding head shape) by generating a phenotypic matrix of variance–covariance (**P** matrix) for each cross type. We first compared the cross types on the basis of each component of **P** (trait variances and trait correlations), then on the general properties of **P** (matrix size, eccentricity and angle), and finally by assessing through Krzanowski's common subspaces method [60] whether parts of **P** (i.e. particular suits of covarying traits) differed in variance.

Within **P**, trait variance and correlation structures of each cross type revealed higher variance in growth after exogeneous feeding in the SB × SB offspring than in the PL × PL offspring (Table 4). More dissimilarity was observed in the hybrids, which were associated with reduced variances in size and growth (from hatching to the onset of first feeding) compared to the two pure-morph offspring. We did not report evidences for differences in trait correlations between cross types (Table 4).

The differences in variance also appeared as trends at the scale of **P** matrices, V_{tot} tending to be the largest in the SB × SB offspring and the smallest in the hybrids

Table 4 Posterior modes and 80% CrIs credible intervals (CrIs) of trait variance that showed nonoverlaps in CrIs between at least two cross types (all CrIs of trait correlations overlapped)

Trait	PL × PL	SB × SB	F_1 hybrids
Standard length (D1)*	0.14 [0.11; 0.18]	0.20 [0.15; 0.27]	0.08 [0.07; 0.10]
Standard length (D3)†	0.21 [0.14; 0.26]	0.21 [0.15; 0.28]	0.10 [0.08; 0.13]
Growth from D1 to D3†	0.18 [0.15; 0.24]	0.22 [0.18; 0.33]	0.13 [0.11; 0.16]
Growth from D3 to D4†	0.31 [0.23; 0.42]	0.68 [0.52; 1.12]	0.42 [0.32; 0.57]
Yolksac-relative area (D1)	0.15 [0.11; 0.18]	0.22 [0.15; 0.27]	0.11 [0.09; 0.14]
Yolksac-conversion	0.15 [0.12; 0.18]	0.21 [0.16; 0.29]	0.12 [0.10; 0.15]

D1 = hatching, D2 = 20 days post hatching, D3 = onset of exogeneous feeding, D4 = 3 months after the onset of exogeneous feeding

* 95% CrI also nonoverlapping

† 90% CrI also nonoverlapping

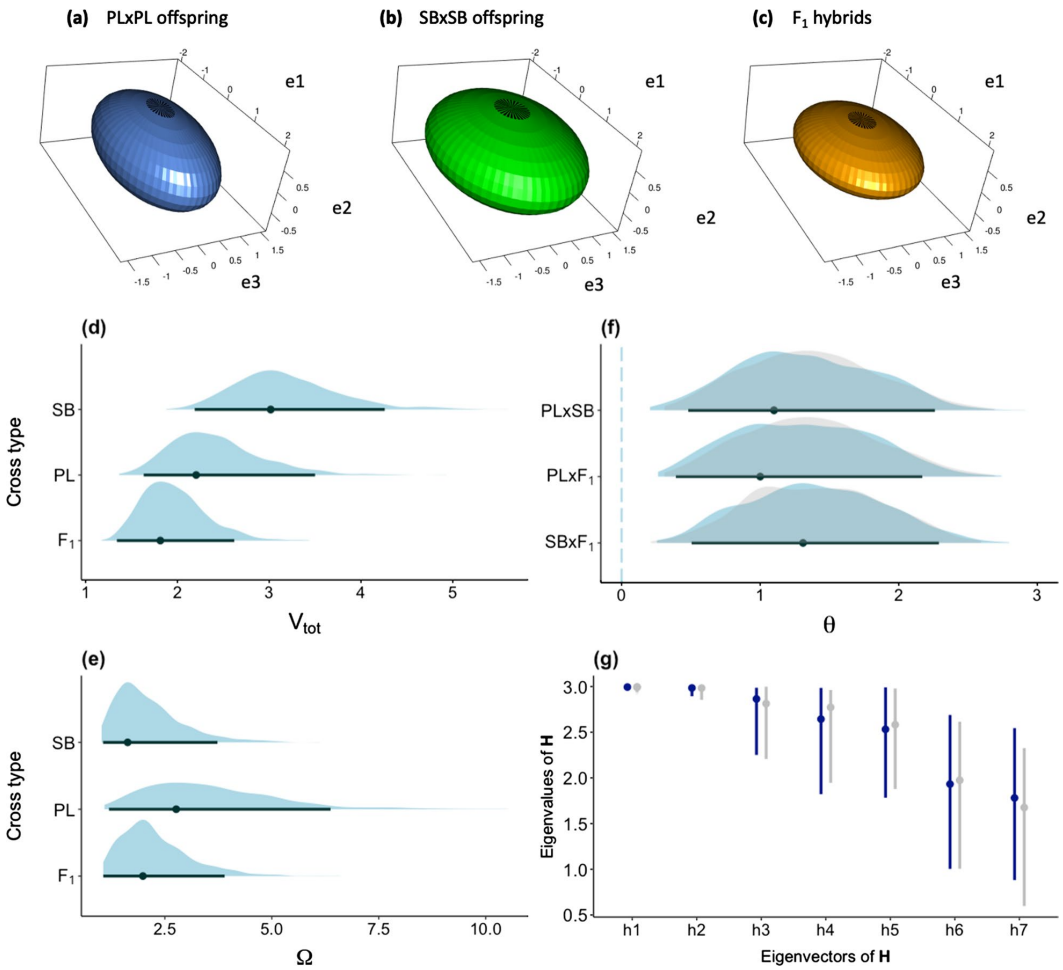

Fig. 4 Summary properties the **P** matrices of each cross type. **a**–**c** Ellipsoid representations of the posterior modes of each matrix projected onto a subspace defined by the first three eigenvectors of **P** from the PL × PL cross. The axes explain 80%, 69% and 77% of the variance of **P** in the PL × PL, the SB × SB and the hybrid crosses, respectively. **d**–**f** Posterior densities, posterior modes and 95% CrIs of the three summary estimates of the matrices of phenotypic variance of each cross type, being **d** the overall phenotypic variance (V_{tot}), **e** the eccentricity (Ω), and **f** the angle (θ) between the first eigenvectors. Densities of the angle estimates between **P** of a cross type (from top to bottom: PL × PL, PL × PL, SB × SB) and a random matrix are shown in light grey. Cross type: SB = SB × SB offspring, PL = PL × PL offspring, F_1 = hybrids. **g** Posterior modes and 95% CrIs of **H** for the observed data (dark blue) and the randomized P matrices (light grey)

(Fig. 4a–c). This indicates higher overall phenotypic variation in the SB × SB than in the PL × PL and the hybrids. A trend for more phenotypic constraints (more eccentricity) also appeared in the PL × PL offspring. However, high uncertainty was associated with the estimates of matrix size and eccentricity (Fig. 4d, e). We did not detect differences in matrix orientation (Fig. 4f), and we did not uncover difference in parts of the trait space through the common-subspace analysis (Fig. 4g).

Correlations between shape and univariate measurements

We estimated differences among cross types in the propensity of head shape (multivariate data) to covary with the variables in the **P** matrices through two-Blocks Partial Least Squares analyses (2B-PLS) [61].

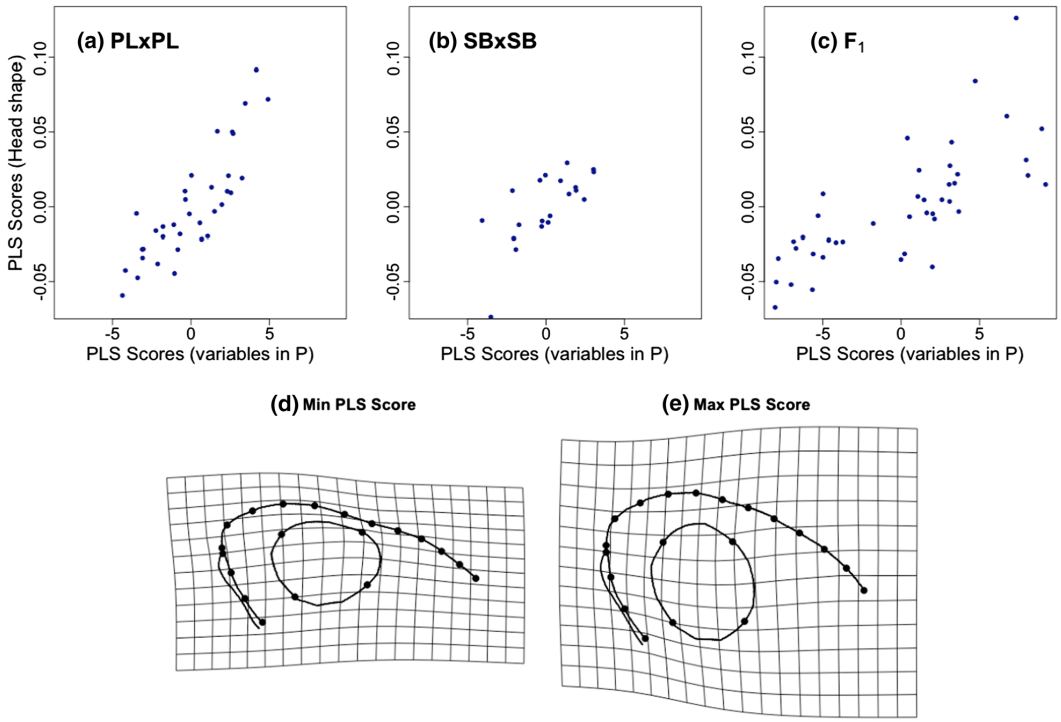

Fig. 5 Results of 2-B PLS analysis of head shape and the variables constituting **P**. **a** PL × PL offspring, **b** SB × SB offspring and **c** hybrids. Deformation grids (**d**, **e**) depict the predicted head shapes in the PL × PL offspring at the extreme PLS scores of the block containing the variables of P

Table 5 First singular vector of the first block (variables in P) from each 2-Block Partial Least Square analysis (one per cross type)

	PL × PL	SB × SB	F1
Standard length at D1	− 0.19	− 0.09	− 0.17
Standard length at D3	− 0.94	0.74	− 0.66
Growth D1 to D2	0.00	0.00	0.00
Growth D3 to D4	− 0.02	− 0.01	− 0.01
Yolk sac relative size at D1	− 0.01	− 0.02	− 0.04
Yolk sac conversion rate	− 0.09	0.49	0.73
Feeding latency	0.28	− 0.46	0.05

Table 6 Two sample Z-scores between pairs of effect sizes (Z) from the cross type specific 2-Block Partial Least Analyses and associated p-values

Cross types	Z[a]	p-value
PL × PL − SB × SB	2.19	0.03
PL × PL-F_1	0.08	0.93
SB × SB-F_1	2.18	0.03

[a] Cross types effect sizes: PL × PL = 3.19; SB × SB = 0.63; F_1 = 3.85

The 2-B PLS analyses revealed high correlations in all three cross types between head shape and the variables of **P** (PL × PL: $PLS_{corr} = 0.84$, $P < 0.001$; SB × SB: $PLS_{corr} = 0.70$, $P = 0.044$, F_1: $PLS_{corr} = 0.75$; $P < 0.001$). Together with the shape change grids, the loadings of the first singular vectors indicate that shape changes are mostly associated with the standard length at the onset of exogeneous feeding (Fig. 5, Table 5). However, the strength of the correlation appeared to be lower in SB × SB offspring than the two other cross types, the effect sizes of the PLS analyses being significantly lower in the SB × SB offspring than in the PL × PL offspring and the hybrids (Table 6). Note that removing the SB × SB individual with the lowest head shape PLS score (although not identified as an outlier in the preliminary analyses) made the pairwise differences nonsignificant (results not shown). Cross type-specific

wireframes thin-plate spline deformation grids describing shape changes at the extremes the of PLS axis are shown in Additional file 1: Figure S2.

Discussion

In our common-garden study, the F_1 hybrids of two sympatric morphs of Artcic charr showed subtle phenotypic differences with the offspring of the two pure morph crosses. First, while SB and PL charr differed in their growth trajectories (which is in line with previous findings about their life-history strategies, Jonsson et al. [52]), the hybrids differed from the PL charr in their growth (although no difference between the hybrids and the SB charr were observed). However, our results did not provide strong evidence for differences between cross types in average values of yolk sac size and resorption. However, head morphology was dependent on size in the same way for the three cross types (common allometry). The juveniles of the two morphs may therefore differ in shape because of their differences in growth. The PL charr also show higher individual consistency in their propensity to start feeding and tended to be less active and less variable in their feeding behaviour than the SB charr, which is in line with previous observations suggesting that the two morphs have evolved different foraging strategies [57].

The lack of evidence for size-independent head shape variations among cross types contrasts with previous observations of differences between PL and SB embryos in the morphology of craniofacial cartilage elements [55]. These differences might be too subtle to be observed on live specimens in lateral view, and the major morphological differences observed between PL and SB charr might also developed at a later developmental time point than in our study. External, size-independent shape differences have been reported between PL × PL and SB × SB offspring 4 to 6 month after the onset of exogeneous feeding [62]. This age might correspond to a period when their wild conspecifics undergo or have already completed ontogenetic niche shift [63]. Unfortunately, information on the exact timing of the ontogenetic niche shift is lacking, and there are to our knowledge no other appropriate experiments on SB and PL charr at earlier stages to shed light on our results.

Overall, we did not observe evidence of multivariate trait divergence between the two Artic charr morphs. The PL × PL and the SB × SB offspring differed in average value for some traits (especially size and growth), but did not show clearly distinct trait variance–covariance structures. Besides, most of the studied traits appeared uncorrelated. Under multivariate divergent selection, the evolutionary trajectories of populations are expected to be biased in the direction of the phenotypic space with the largest variance (i.e. "lines of least resistance" [64]). These trajectories may be even more complicated by various parameters like the direction of correlational selection relative to the trait with greatest genetic variance, the strength of genetic correlations, the frequency of hybridization and the fitness of hybrids [65, 66]. Genetic covariances and correlations might especially facilitate adaptive changes but also constrain them [67, 68]. In our study, the lack of putative evidence for genetically based trait correlations and the apparent homogeneity of variance among traits (implying the absence of Schluter's "line of least resistance") suggest that no evolutionary constraint complicates the divergence of the two morphs. Note, however, that we treated the eggs clutches as a fixed effect when generating the P matrices, because of our limited number of families. Thus, the variance component related to family effects and early environmental variations could not be estimated through variance partitioning. Therefore, our results need to be carefully interpreted considering that these important aspects of phenotypic variation were corrected for but not quantified.

We did not find differences in average trait values that would imply substantial fitness consequences in wild F_1 hybrids. The hybrids from our study were not strictly intermediate nor transgressive but rather show parental bias (e.g. were similar to the SB × SB offspring in their growth, yolk sac resorption and feeding behaviour). Because the two hybrid cross types were pooled for the analyses, we were not able to test for differences between reciprocal hybrids nor to assess whether one type of hybrids accounted for most of the parental bias. Still, this observation is contrasting with other common-garden experiments reporting intrinsic developmental deficiencies or transgressive characters with obvious ecological implications in F_1 hybrids between recently diverged populations [37, 69]. For example, hybrids between sympatric charr morphs of Lake Sobachye (Taimyr) develop detrimental ossification anomalies [70], and higher mortalities but intermediate hatching dates were observed in hybrids between lake whitefish ecotypes, *Coregonus cluteaformis* [28]. Considering the parental bias in average trait values observed here and the putative absence of trait correlations, hybrid disadvantages might be occurring (if ever) as functional mismatches. Trait mismatches consist in novel combinations of independent traits with non-intermediate values [71] and may often occur in F_1 hybrids because of the common effects of dominance [30]. Such functional mismatches also appear plausible in light of the highly numerous regions of differentiation scattered across the entire PL and SB charr genomes [50], suggesting that a diverse suit of traits might have evolved in response to divergent selection.

The lower variance for growth traits observed in the hybrids goes contrary to our predictions of increased phenotypic variability through hybridization. Together with growth in hybrids being as low as in the smallest morph, these observations might be the only hints of developmental deficiencies in the hybrids. Growth-related traits are known for often being highly related to fitness [72, 73], so one may expect slow and lowly variable growth to impact the ecology of hybrids. Of course, consequential developmental unviability as well as novel phenotypes and enhanced phenotypic variability may occur in recombinant (F_2) and backcrossed hybrids, as observed in many systems [28, 38, 71, 74, 75]. Differences in ecologically relevant traits might also be detectable at later developmental time points than those covered by our study; the ontogenetic niche shift between the two morphs probably occurring as late as several months after the onset of exogeneous feeding [63]. Further studies on later generations of hybrids—although highly constraining regarding the life cycle and the elusive behaviour of the species—may shed more light on the implication of hybridisation regarding postzygotic isolation or phenotypic diversification.

Our results provided little support to the hypothesis of intrinsic postzygotic isolation between the PL and the SB charr (i.e. reproductive barrier produced by environment-independent hybrid deficiencies). Moreover, the singularity of hybrids in terms of average trait values and trait covariance suggests that selection against hybrids might be effective, although these observed differences were subtle, and their fitness consequences are unknown. Thus, the question of reproductive isolation in the two charr remains unresolved. In a recent study on the genetic structure of the two charr, about ten percent of the fishes were identified as potential hybrids [76], so substantial though incomplete reproductive barriers must have evolved between these sympatric morphs and are yet to be discovered. Combined with research on assortative mating and on fine-scale spatiotemporal segregation during spawning, studying the fitness cost of the hybrid characters described above would constitute a promising approach to unravel the evolutionary origins of the Arctic charr morphs of Thinvallavatn.

Conclusion

Increased trait dimensionality is expected to facilitate local adaptation, sometimes to such an extent that phenotypic divergence can easily occur in the face of high gene flow [66]. Although this should be expected in the SB and the PL charr that seem to be under divergent selection for various trophic and non-trophic traits [77], we did not observe strong evidence for multivariate phenotypic divergence through an extensive phenotypic survey covering different ontogenetic stages. The strongest signal of genetically based differentiation came from growth, which covaried with morphology but not with other traits. Therefore, the divergence of the two morphs might occur without substantial evolutionary constraints nor facilitations. Whether such trend is commonplace or not remains to be established. Northern freshwater fish would be highly suitable model to explore this view. Numerous diverging populations with diverse evolutionary histories, phenotypic distances and reproductive diversification are being extensively studied on the ecological, the genetic and the genomic grounds [42, 43, 78], which now provide consequential resources for multivariate studies on the ontogeny of hybrid phenotypes.

Methods

Study system

Thingvallavatn is a deep postglacial lake (surface 84 km^2, mean depth: 34 m) that formed within a graben of the Mid-Atlantic ridge during the last glacial retreat (*ca.* 10,000 years BP) [79, 80]. The lake is characterized by a wide pelagic zone and three major benthic habitats: a "stony littoral" zone (0–10 m deep) composed of a spatially complex lava substrate with loose stones, crevasses and interstitial spaces, a deeper zone (10–20 m deep), densely vegetated by the algae *Nitella opaca*, and a profundal zone (25 m and deeper) covered by a diatomic gyttja substrate [53, 81]. The lake hosts four morphs of Arctic charr. Two of them, the planktivorous (PL) and the piscivorous charr (PI) feed in the pelagic and epibenthic layers, respectively, and are characterised by a terminal mouth and relatively small pectoral fins [82]. The two other morphs, the large-benthic (LB) and the small-benthic charr (SB), forage in the benthic zone, and show a blunt snout with a subterminal mouth and large pectoral fins [51–53]. The PL and the SB charr are currently found exclusively in sympatry, although coalescent simulations supports evolutionary scenarios involving short periods of geographic isolation [48]. The differentiation of the craniofacial morphology among the two morphs is initiated early during development, before hatching [55], but can also be influenced to some extent by plasticity after the onset of exogeneous feeding [62]. The SB charr spawn from August to November and the PL charr from September to October [58]. The young of the year of the two morphs are believed to use the same habitat, the surf zone (0–1 m deep), from the onset of active feeding in the spring until the summer, when the PL-charr are thought to migrate towards the pelagic and the epibenthic zones [63].

Fish collection and rearing

We collected mature SB and PL charr with gillnets during five sessions of night fishing in October 2017, at a single spawning site known to be used by both morphs (Svínanesvík, 64° 11′ 24.6″ N; 21° 05′ 40.5″ W; [58]). We used 52 fish to generate 26 full-sib families on site (crossing design in Additional file 1: Table S4). The eggs were kept at 4.1 ± 0.2 °C in a vertical incubator (Mari-Source, USA). On the mean hatching day (when 50% of the embryos from a given family had hatched), 40 free-swimming embryos from each one of the first nine families to hatch were moved into single-individual cylinders with a plastic mesh on the lower side to allow water flows (2.2 cm diameter × 6.0 cm height, 0.1 cm^2 mesh size), and placed into a EWOS tray (60 × 250 cm) with flow-through water. All families and cross types hatched at a similar developmental time point (Additional file 1: Figure S4). Before first feeding (ca. 530 degree days—°C d, March 2018), embryos were moved into 22 cl transparent plastic cups placed in the same EWOS tray (6.1 ± 0.6 °C). These cups were perforated on the sides and were assumed to enable the exchange of olfactory cues and visual contact between congeners. The cups were weekly shuffled inside the setup to overcome eventual confounding effects caused by heterogeneous physical parameters. The fish were fed ad libitum two or three times a day with ground aquaculture pellets (Inicio Plus G 0.4 mm, BIOMAR).

Data collection

We measured the craniofacial development, pre- and post-feeding growth, and yolk-sac resorption using morphometric data from photographs taken at four points throughout ontogeny: at hatching (ca. 445 °C d), 20 days post-hatching (ca. 530 °C d), 3 to 4 weeks after the onset of exogeneous feeding (ca. 840 °C d) and 9 to 11 weeks after the onset of exogeneous feeding (ca. 1100 °C d). The fish were anaesthetized with 2-phenoxyethanol [83], positioned on their lateral side facing left and photographed with a fixed, down-facing camera (Canon EOS 650D + 100 mm macro lens) before being returned to their respective growing cell. To correct for the tilt caused by the yolk-sac, the specimens were positioned on 3% methyl cellulose [84] for the photographs of the first two timepoints.

The timing of the onset of exogeneous feeding was determined through "One-zero" sampling (i.e. records of the occurrence or non-occurrence of an event within defined observation periods) [59]. Direct observations were made every day on all fish, starting when food was introduced in the rearing setup for the first time (ca. 635 °C d). This was done in the following way: a 3-min observation trial was initiated on each focal individual as the observer introduced food (ca. 10 slowly sinking ground pellets particles of 0.4 mm or less) into the cup of the focal fish. We determined the date of the onset of exogeneous feeding as the date the focal fish was observed catching food for the first time.

Several key aspects of feeding behaviour were estimated by conducting three focal sampling sessions [59] over 3 consecutive days, 7 days after the date of first feeding of the focal individual. We measured behaviours involved in food particle snapping, which constitute a convenient way to study foraging behaviour in captive Arctic charr juveniles [17, 21]. Differences in these behavioural variables were observed between Arctic charr of contrasting sizes (from an aquaculture strain) several weeks after the onset of exogeneous feeding [17]. A 3 min observation period was initiated following the introduction of the food, to record the time it took the fish to seize the first particle (reaction time) [17]. From this point on, an extra 1-min observation trial was initiated, during which feeding intensity (number of particles caught) and feeding strategy (proportion of particles caught on the bottom, on the surface and in mid-water) were recorded. The focal fish was considered "nonfeeding" and the trial was terminated if no particle was seized by the end of the initial 3-min observation period. The observer was not aware of the cross type of the focal individual when conducting the observation trial.

Digitizing and pre-processing morphological data

Data on size (standard lengths) and morphology were extracted from photographs using Geometric morphometrics methods [85]. We placed landmarks on the tip of the lower jaw, the lower edge of the maxilla below the centre of the eye, the point of maximum curvature between the brain and the cranium, the extremity of the notochord and the anus (Additional file 1: Figure S3). We digitized the contours of the eye, of the head (from the lower edge of the maxilla below the centre of the eye to the point of maximum curvature between the brain and the cranium) and of the yolk sac (from the junction with the vitellin vein to posterior junction with the body) with Bezier curves using the R package Stereomorph. During the standard pre-processing steps (i.e. superimposing the landmark configurations of all specimens to a common coordinate system through Generalized Procrustes Analysis) [86], we estimated the surface of the yolk sac as the area of a polygon composed of 200 semi-landmarks extracted from its respective curve. We calculated the standard length of all specimens as the Euclidian distance between the

extremity of the notochord and the furthest of 50 semi-landmarks generated from the curve along the head. The dataset used for the analyses of head shape consisted in 20 landmarks (the 3 initial landmarks located on head, plus 13 and 4 semi-landmarks extracted for the curves around the head and the eye, respectively).

Analyses of individual traits

We modelled the growth trajectories of every specimen in each cross type using polynomial random regressions [87]. We then tested for overall differences between cross type in the development of the head by conducting phenotypic trajectory analyses of the Procrustes residuals of the head [86]. Morphological disparity analyses [88] were used to compare the types of crosses on the basis of within-group variations in head shapes at the third developmental time-point (3–4 weeks after first feeding). We also tested for group differences in the date of first feeding, feeding intensity, and foraging behaviour with separate GLMMs. The specifications of each model are described in Additional file 1: Table S5. Although reciprocal hybrid crosses were made (numbers in Additional file 1: Table S4), we pooled the hybrids of both maternal origins in the GLMMs to gain sufficient statistical power.

All the GLMMs were run with the R package MCMCglmm [89]. MCMCglmm relies on a Bayesian framework using Markov chain Monte Carlo (MCMC) methods. We always set weakly informative priors ($V=1$, $nu=0.002$ or the number of traits for the multi-response models) and determined the optimal number of iterations for model convergence through the examination of trace plots, posterior density plots and effective sample sizes (Additional file 1: Table S5). Inferences were made by comparing the posterior mode estimates and 95% Highest Posterior Density Credible intervals (95% CrI) of each cross type (and in relation to the zero baseline for the significance of R estimates).

We studied between-individual variations in feeding behaviour by comparing repeatability estimates among the three cross types. The repeatability of each behavioural variable measured across the three repeated observational trials (propensity to start feeding, number of caught items, vertical location) was calculated according to the formula of adjusted repeatability in [90]. The repeatability estimates of the propensity to start feeding, a variable with binary data, were calculated accounting for Jensen's inequality when transforming the results (initially on the latent scale) to the data scale, following [91].

Trait covariance

We studied the patterns of trait covariance by generating a phenotypic matrix of variance–covariance (**P** matrix) for each cross type. **P** matrices are reliable surrogates of genetically based patterns of trait covariances (i.e. of the **G** matrices) when no pedigree is available [64, 92]. **P** matrices are especially likely to be good proxies in our particular study because the effects of the environment were mitigated by the use of common-garden conditions, and because the parental effects were accounted for by including in the subsequent models the family of origin (i.e. the egg clutch) of all individuals. We estimated the components of the three matrices by running three separate Multi-Response Generalized Mixed models [89]. All three models contained seven variables as a response (Table 1). The family was included as a fixed effect while the identity of the individual was included as a random factor. All the traits were mean-standardized by dividing the raw values by their group means [93].

The **P** matrices of each cross type were first compared on the basis of their size, shape and orientation [94]. The matrices sizes (V_{tot}) were used to compare the types of crosses in the overall phenotypic variance and were calculated as the sum of their eigenvalues (Eq. 2 in [95]) [94, 95]. Eccentricity (Ω) was used as a measure of the shape of the matrices and was calculated as the ratio of the first two eigenvalues [94]. Differences in overall matrix orientation were assessed using the angles (θ) between the first eigenvector of each **P** matrix. Briefly, if the patterns of trait covariances were not conserved but have rapidly evolved among the two morphs, we expected the two types of pure-morph offspring to show differences in the overall size of **P** (V_{tot}), which should suggest a response to two selective regimes eroding genetic variations to different extents. Similarly, differences in eccentricity (Ω) between the two purebred offspring were expected (for example, correlational selection, which can produce more constrained, "cigar shaped", **G** matrices [94], might differ among the respective habitats of each morph). The orientation of **G** can also be subjected to changes because of the effects of correlational selection, among other evolutionary forces [94, 96, 97]. Thus, differences between purebred offspring in the orientation of **P** (θ) were also expected [68]. Regarding the hybrids, breakdowns in their trait covariance structure should be indicated by **P** matrices with larger sizes and reduced eccentricity [38]. Meanwhile, differences in the orientation of **P** between the hybrid and the purebred offspring should indicate whether the remaining constraints on the hybrid phenotypes are intermediate, under dominance and conserved relative to one morph, or transgressive (i.e. biased toward a unique direction of the phenotypic space).

Next, we assessed which part of **P** (i.e. which suits of covarying traits) differed the most among cross types in their variance by using Krzanowski's common subspaces method [60]. This method produces a set of vectors (**H**) that can be used to determine the groups'

similarities in parts of the trait space. Eigenvalues of **H** indicates the degree of resemblance between principal components of the trait subspaces of each group while the eigenvectors are informative of the variables associated with this resemblance. We used the approach of [98], which implements the subspace method in a Bayesian framework. Eigenvalues tending towards the number of measured variables would indicate highly similar subspaces. Significance was assessed through a comparison with eigenvalues generated by randomized **P** matrices (by randomly assigning individuals of each cross types to three groups).

For visualisation purposes, **P** matrices were projected into a subspace composed by the first three eigenvectors **P** matrix of the PL × PL offspring by modifying the plotsubspace() function from [89]. Because angles between eigenvectors are necessarily positive, we compared the angles between the first eigenvectors of **P** with the angles between the first eigenvector of one cross type (depending on the comparison) and the first eigenvector of a "random" **P** matrix. The simulated matrix was generated by sampling 150 individuals from the two cross types being compared.

Covariance between head shape and univariate traits

Because of the complex multivariate nature of shape data, univariate proxies of shape changes were not used to generate the **P** matrices. Instead, we relied on Two-Blocks Partial Least Squares (2B-PLS) analyses [61] to assess the propensity of head shape at the onset of exogeneous feeding to covary with the variables constituting the **P** matrices. We relied on the method of Adams and Collyer [99] for pairwise comparisons among cross types in the correlation between shape and the other variables. For these analyses only, the latency to first feeding (initially the three measurements per individual) was averaged.

Abbreviations

F_1: Cross of first generation (here mainly refers to first-generation hybrids); GLMM: Generalized Linear Mixed-effect Model; 2-B PLS: 2-Bocks Partial Least-Square analysis; PL: Planktivorous charr; PL × PL: Cross produced with the gametes of two planktivorous charr; SB: Small benthic charr; SB × SB: Cross produced with the gametes of two small benthic charr; 95% CrI: 95% Highest Posterior Density Credible intervals.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12862-021-01904-8.

Additional file 1. Additional Tables and Figures.

Additional file 2. Datsets.

Acknowledgements

We are very thankful to Skúli Skúlason for his comments of the manuscript and to Neil Metcalfe for his advice for analysing the data on growth and development. We are also thankful to David Benhaïm and Bjarni K. Kristjánsson for their comments on the experimental design and the conceptual aspects of the study. Field work could be conducted thanks to the help of Zophonías O. Jónsson, the members of the Arctic Charr and Salmonids Group of the University of Iceland, and the owners of the farm of Mjóanes, Jóhann Jónsson and Rósa Jónsdóttir. We thank Kári H. Árnason, Rakel Þorbjörnsdóttir, and Christian Beuvard for the organisation and the maintenance of the experimental setup. We are also thankful to Alia Desclos for her help in rearing the fish.

Authors' contributions

QJH conceived the study, reared the specimens, collected the data, conducted the analyses and drafted the manuscript. SSS coordinated the field work, produced the embryos and critically revised the manuscript. MBM provided guidance for the data analyses, contributed to the biological interpretations of the results and reviewed the manuscript. CAL developed the rearing setup, contributed in designing the behavioural experiments and reviewed the manuscript. KHK established the crossing design, produced the embryos, organized the logistics of the transfer and the maintenance of the specimens, provided guidance during the experiments and critically revised the manuscript. All authors gave their final approval for publication and agree to be accountable for the work therein. All authors read and approved the final manuscript.

Funding

This work was fully funded by the Icelandic Centre of Research, RANNÍS (Icelandic Research Fund grant no.173802-051). The funding body did not have any role in the design of the study, data collection, the analyses nor in the interpretations of the results.

Availability of data and materials

The datasets supporting the conclusions of this article are included within additional files of the article (Additional file 2). R codes are available at https://github.com/quentin-evo/multi_trait_hybrid_charr/.

Declarations

Ethics approval and consent to participate

Sampling was conducted with the permissions of the owner of the farm of Mjóanes and the Thingvellir National Park commission. Ethics committee approvals for research projects are not required by the Icelandic regulation (Act No. 55/2013 on Animal Welfare). The rearing and the experimental work were conducted in the facilities of Hólar University College, which has an operational license under the Icelandic Aquaculture law (Law No. 71/2008): License number FE-1051 for Verið Research station. This law includes clauses of best practices for animal care and experimental work. The fish were killed according to the most careful euthanasia guidelines for salmonid fishes [83], and the optimal dosage for anaesthesia on 2-phenoxyethanol was adjusted to the reactions of each individual, following the recommendations of the laboratory facility. Decisions on the sample size and on the design of the common-garden experiment were made to ensure that additional studies could be conducted with data collected on the same specimens. The study conformed to the ARRIVE guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 11 May 2021 Accepted: 18 August 2021
Published online: 07 September 2021

References

1. Rundle HD, Nosil P. Ecological speciation. Ecol Lett. 2005;8:336–52.
2. Nosil P. Ecological speciation. 1st ed. Oxford: Oxford University Press; 2012.
3. Coyne JA, Orr AH. Speciation. 1st ed. Sunderland: Sinauer Associates, Inc.; 2004.
4. Schluter D. Ecological speciation in postglacial fishes. Philos Trans R Soc B Biol Sci. 1996;351:807–14. https://doi.org/10.1098/rstb.1996.0075.
5. Hendry AP. Ecological speciation! Or the lack thereof? Can J Fish Aquat Sci. 2009;66:1383–98. https://doi.org/10.1139/F09-074.
6. Seehausen O, Butlin RK, Keller I, Wagner CE, Boughman JW, Hohenlohe PA, et al. Genomics and the origin of species. Nat Rev Genet. 2014;15:176–92. https://doi.org/10.1038/nrg3644.
7. Higham TE, Rogers SM, Langerhans RB, Jamniczky HA, Lauder GV, Stewart WJ, et al. Speciation through the lens of biomechanics : locomotion, prey capture and reproductive isolation. Proc R Soc B. 2016. https://doi.org/10.1098/rspb.2016.1294.
8. Roff DA, Fairbairn DJ. The evolution of trade-offs: where are we? J Evol Biol. 2007;20:433–47.
9. Stearns SC. Trade-offs in life-history evolution. Funct Ecol. 1989;3:259–68. https://doi.org/10.2307/2389364.
10. Arnold SJ. Constraints on phenotypic evolution. Am Nat. 1992;140:85–107.
11. Saltz JB, Hessel FC, Kelly MW. Trait correlations in the genomics era. Trends Ecol Evol. 2017;32:279–90. https://doi.org/10.1016/j.tree.2016.12.008.
12. Kern EMA, Robinson D, Gass E, Godwin J, Langerhans RB. Correlated evolution of personality, morphology and performance. Anim Behav. 2016;117:79–86. https://doi.org/10.1016/j.anbehav.2016.04.007.
13. Sinervo B, Svensson E. Correlational selection and the evolution of genomic architecture. Heredity (Edinb). 2002;89:329–38.
14. Beck SV, Räsänen K, Ahi EP, Kristjánsson BK, Skúlason S, Jónsson ZO, et al. Gene expression in the phenotypically plastic Arctic charr (*Salvelinus alpinus*): a focus on growth and ossification at early stages of development. Evol Dev. 2018;21:16–30.
15. Jardine D, Litvak MK. Direct yolk sac volume manipulation of zebrafish embryos and the relationship between offspring size and yolk sac volume. J Fish Biol. 2003;63:388–97.
16. Leblanc CAL, Kristjánsson BK, Skúlason S. The importance of egg size and egg energy density for early size patterns and performance of Arctic charr *Salvelinus alpinus*. Aquac Res. 2014;47:1–12.
17. Leblanc CAL, Benhaïm D, Hansen BR, Kristjánsson BK, Skúlason S. The importance of egg size and social effects for behaviour of Arctic charr juveniles. Ethology. 2011;117:664–74.
18. Ferrari MCO, Brown GE, Bortolotti GR, Chivers DP. Prey behaviour across antipredator adaptation types: how does growth trajectory influence learning of predators? Anim Cogn. 2011;14:809–16.
19. Adams CE, Woltering C, Alexander G. Epigenetic regulation of trophic morphology through feeding behaviour in Arctic charr, *Salvelinus alpinus*. Biol J Linn Soc. 2003;78:43–9.
20. Wimberger PH. Plasticity of fish body shape. The effects of diet, development, family and age in two species of *Geophagus* (Pisces: Cichlidae). Biol J Linn Soc. 1992;45:197–218.
21. Benhaim D, Skúlason S, Hansen BR. Behavioural variation in juvenile Arctic charr in relation to body size. J Fish Biol. 2003;62:1326–38.
22. Biro PA, Stamps JA. Are animal personality traits linked to life-history productivity? Trends Ecol Evol. 2008;23:361–8.
23. Polverino G, Santostefano F, Díaz-Gil C, Mehner T. Ecological conditions drive pace-of-life syndromes by shaping relationships between life history, physiology and behaviour in two populations of Eastern mosquitofish. Sci Rep. 2018;8:1–10.
24. Nosil P, Harmon LJ, Seehausen O. Ecological explanations for (incomplete) speciation. Trends Ecol Evol. 2009;24:145–56.
25. Sobel JM, Chen GF, Watt LR, Schemske DW. The biology of speciation. Evolution (N Y). 2010;64:295–315.
26. Riesch R, Tobler M, Lerp H, Jourdan J, Doumas T, Nosil P, et al. Extremophile Poeciliidae: multivariate insights into the complexity of speciation along replicated ecological gradients. BMC Evol Biol. 2016;16:1–15. https://doi.org/10.1186/s12862-016-0705-1.
27. Grant PR, Grant BR. Phenotypic and genetic effects of hybridization in Darwin's finches. Evolution (N Y). 1994;48:297–316.
28. Rogers SM, Bernatchez L. The genetic basis of intrinsic and extrinsic post-zygotic reproductive isolation jointly promoting speciation in the lake whitefish species complex (*Coregonus clupeaformis*). J Evol Biol. 2006;19:1979–94.
29. Santos-Santos JH, Audenaert L, Verheyen E, Adriaens D. Ontogenetic divergence generates novel phenotypes in hybrid cichlids. J Anat. 2021;238:1116–27.
30. Thompson KA, Urquhart-Cronish M, Whitney KD, Rieseberg LH, Schluter D. Patterns, predictors, and consequences of dominance in hybrids. bioRxiv. 2019.
31. Cooper BS, Sedghifar A, Nash WT, Comeault AA, Matute DR. A maladaptive combination of traits contributes to the maintenance of a drosophila hybrid zone. Curr Biol. 2018;28:2940-2947.e6. https://doi.org/10.1016/j.cub.2018.07.005.
32. Dagilis AJ, Kirkpatrick M, Bolnick DI. The evolution of hybrid fitness during speciation. PLoS Genet. 2019;15:1–21.
33. Ackermann RR, Rogers J, Cheverud JM. Identifying the morphological signatures of hybridization in primate and human evolution. J Hum Evol. 2006;51:632–45.
34. Coolon JD, Mcmanus CJ, Stevenson KR, Coolon JD, Mcmanus CJ, Stevenson KR, et al. Tempo and mode of regulatory evolution in *Drosophila*. Genome Res. 2014;24:797–808.
35. McGirr JA, Martin CH. Hybrid gene misregulation in multiple developing tissues within a recent adaptive radiation of Cyprinodon pupfishes. PLoS ONE. 2019;14:e0218899.
36. Renaut S, Nolte AW, Bernatchez L. Gene expression divergence and hybrid misexpression between lake whitefish species pairs (*Coregonus* spp. Salmonidae). Mol Biol Evol. 2009;26:925–36.
37. Mack KL, Campbell P, Nachman MW. Gene regulation and speciation in house mice. Genome Res. 2016;26:451–65.
38. Selz OM, Lucek K, Young KA, Seehausen O. Relaxed trait covariance in interspecific cichlid hybrids predicts morphological diversity in adaptive radiations. J Evol Biol. 2014;27:11–24.
39. Snorrason SS, Skúlason S. Adaptive speciation in northern freshwater fishes. In: Adaptive speciation. 2004. p. 210–28. https://doi.org/10.1017/CBO9781139342179.012.
40. Skúlason S, Smith TB. Resource polymorphisms in vertebrates. Trends Ecol Evol. 1995;10:366–70.
41. Smith TB, Skúlason S. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. Annu Rev Ecol Syst. 1996;27:111–33.
42. Skúlason S, Parsons KJ, Svanbäck R, Räsänen K, Ferguson MM, Adams CE, et al. A way forward with eco evo devo: an extended theory of resource polymorphism with postglacial fishes as model systems. Biol Rev. 2019;94:1786–808.
43. Lackey ACR, Boughman JW. Evolution of reproductive isolation in stickleback fish. Evolution (N Y). 2017;71:357–72.
44. Doenz CJ, Bittner D, Vonlanthen P, Wagner CE, Seehausen O. Rapid buildup of sympatric species diversity in Alpine whitefish. Ecol Evol. 2018;8:9398–412.
45. Østbye K, Hagen Hassve M, Peris Tamayo AM, Hagenlund M, Vogler T, Præbel K. "And if you gaze long into an abyss, the abyss gazes also into thee": four morphs of Arctic charr adapting to a depth gradient in Lake Tinnsjøen. Evol Appl. 2020;13:1240–61.
46. Esin EV, Markevich GN, Pichugin MY. Juvenile divergence in adaptive traits among seven sympatric fish ecomorphs arises before moving to different lacustrine habitats. J Evol Biol. 2018;31:1018–34.
47. Foote AD. Sympatric speciation in the genomic era. Trends Ecol Evol. 2017;33:85–95. https://doi.org/10.1016/j.tree.2017.11.003.
48. Kapralova KH, Morrissey MB, Kristjánsson BK, Olafsdóttir GÁ, Snorrason SS, Ferguson MM. Evolution of adaptive diversity and genetic connectivity in Arctic charr (*Salvelinus alpinus*) in Iceland. Heredity (Edinb). 2011;106:472–87. https://doi.org/10.1038/hdy.2010.161.
49. Kapralova KH, Guðbrandsson J, Reynisdottir S, Santos CB, Baltanás VC, Maier VH, et al. Differentiation at the MHCIIα and Cath2 loci in sympatric *Salvelinus alpinus* resource morphs in Lake Thingvallavatn. PLoS ONE. 2013;8:e69402.
50. Guðbrandsson J, Kapralova KH, Franzdóttir SR, Bergsveinsdóttir TM, Hafstað V, Jónsson ZO, et al. Extensive genetic divergence between recently

evolved sympatric Arctic charr morphs. Ecol Evol. 2019;9:1–20. https://doi.org/10.1101/489104.
51. Snorrason SS, Skulason S, Jonsson B, Malmquist HJ, Jonasson PM, Sandlund OT, et al. Trophic specialization in Arctic charr *Salvelinus alpinus* (Pisces; Salmonidae): morpholigcal divergence and ontogenic niche shifts. Biol J Linn Soc. 1994;52:1–18.
52. Jonsson B, Skúlason S, Snorrason SS, Sandlund OT, Malmquist HJ, Jónasson PM, et al. Life history variation of polymorphic Arctic charr (*Salvelinus alpinus*) in Thingvallavatn, Iceland. Can J Fish Aquat Sci. 1988;45:1537–47.
53. Sandlund OT, Gunnarsson K, Jónasson PM, Jonsson B, Lindem T, Magnússon KP, et al. The Arctic charr *Salvelinus alpinus* in Thingvallavatn. Oikos. 1992;64:305–51.
54. Adalsteinsson H, Jónasson PM, Rist S. Physical characteristics of Thingvallatn, Iceland. Oikos. 1992;64:121–35.
55. Kapralova KH, Jónsson ZO, Palsson A, Franzdóttir SR, le Deuff S, Kristjánsson BK, et al. Bones in motion: Ontogeny of craniofacial development in sympatric Arctic charr morphs. Dev Dyn. 2015;244:1168–78.
56. Parsons KJ, Skúlason S, Ferguson M. Morphological variation over ontogeny and environments in resource polymorphic arctic charr (*Salvelinus alpinus*). Evol Dev. 2010;12:246–57.
57. Skúlason S, Snorrason SS, Ota D, Noakes DLG. Genetically based differences in foraging behaviour among sympatric morphs of Arctic charr (Pisces: Salmonidae). Anim Behav. 1993;45:1179–92.
58. Skúlason S, Snorrason SS, Noakes DLG, Ferguson MM, Malmquist HJ. Segregation in spawning and early life history among polymorphic Arctic charr, *Salvelinus alpinus*, in Thingvallavatn, Iceland. J Fish Biol. 1989;35:225–32.
59. Altmann J. Observtional study of behavior: sampling methods. Behaviour. 1973;49:227–66.
60. Krzanowski WJ. Between-groups comparison of principal components. J Am Stat Assoc. 1979;74:703–7.
61. Rohlf FJ, Corti M. Use of two block partial least squares to study covariation in shape. Syst Biol. 2000;49:740–53.
62. Parsons KJ, Sheets HD, Skúlason S, Ferguson MM. Phenotypic plasticity, heterochrony and ontogenetic repatterning during juvenile development of divergent arctic charr (*Salvelinus alpinus*). J Evol Biol. 2011;24:1640–52.
63. Sandlund OT, Malmquist HJ, Jonsson B, Skúlason S, Snorrason SS, Jónasson PM, et al. Density, length distribution, and diet of age-0 Arctic charr *Salvelinus alpinus* in the surf zone of Thingvallavatn, Iceland. Environ Biol Fishes. 1988;23:183–95.
64. Schluter D. Adaptive radiation along genetic lines of least resistance. Evolution (N Y). 1996;50:1766.
65. Guillaume F, Whitlock MC. Effects of migration on the genetic covariance matrix. Evolution (N Y). 2007;61:2398–409.
66. MacPherson A, Hohenlohe PA, Nuismer SL. Trait dimensionality explains widespread variation in local adaptation. Proc R Soc B Biol Sci. 2015;282:1–8.
67. Agrawal AF, Stinchcombe JR. How much do genetic covariances alter the rate of adaptation ? Proc R Soc B. 2009;276:1183–91.
68. Royauté R, Hedrick A, Dochtermann NA. Behavioral syndromes shape evolutionary trajectories via conserved genetic architecture. Proc R Soc B Biol Sci. 2020. https://doi.org/10.1101/619411.
69. Stelkens RB, Schmid C, Selz O, Seehausen O. Phenotypic novelty in experimental hybrids is predicted by the genetic distance between species of cichlid fish. BMC Evol Biol. 2009;9:1–13.
70. Pichugin MY. The development of an artificial hybrid and revealing elements of reproductive isolation between sympatric forms of Dryagin's char and Salvelinus alpinus complex (Salmonidae) from Sobachye Mountain Lake (Taimyr). J Ichthyol. 2009;49:236–48.
71. Arnegard ME, McGee MD, Matthews B, Marchinko KB, Conte GL, Kabir S, et al. Genetics of ecological divergence during speciation. Nature. 2014;511:307–11.
72. Metcalfe NB, Monaghan P. Compensation for a bad start: grow now, pay later? Trends Ecol Evol. 2001;16:254–60.
73. Metcalfe NB, Van Leeuwen TE, Killen SS. Does individual variation in metabolic phenotype predict fish behaviour and performance? J Fish Biol. 2016;88:298–321.
74. Albertson RC, Kocher TD. Genetic architecture sets limits on transgressive segregation in hybrid cichlid fishes. Evolution (N Y). 2005;59:686–90.
75. Feller AF, Selz OM, McGee MD, Meier JI, Mwaiko S, Seehausen O. Rapid generation of ecologically relevant behavioral novelty in experimental cichlid hybrids. Ecol Evol. 2020;10:7445–62.
76. Brachmann MK, Parsons K, Skúlason S, Ferguson MM. The interaction of resource use and gene flow on the phenotypic divergence of benthic and pelagic morphs of Icelandic Arctic charr (*Salvelinus alpinus*). Ecol Evol. 2021;11:7315–34.
77. Franklin OD, Skúlason S, Morrissey MB, Ferguson MM. Natural selection for body shape in resource polymorphic Icelandic Arctic charr. J Evol Biol. 2018;31:1498–512.
78. Bernatchez L, Renaut S, Whiteley AR, Derome N, Jeukens J, Landry L, et al. On the origin of species: Insights from the ecological genomics of lake whitefish. Philos Trans R Soc B Biol Sci. 2010;365:1783–800.
79. Pétursson HG, Norðdahl H, Ingólfsson O. Late Weichselian history of relative sea level changes in Iceland during a collapse and subsequent retreat of marine based ice sheet. Cuad Investig Geográfica. 2015;41:261–77.
80. Sæmundsson K. Geology of Thinvallavatn area. Oikos. 1992;64:40–68.
81. Sandlund OT, Jonsson B, Malmquist HJ, Gydemo R, Lindem T, Skúlason S, et al. Habitat use of arctic charr *Salvelinus alpinus*. Environ Biol Fishes. 1987;20:263–4.
82. Snorrason SS, Skúlason S, Sandlund OT, Malmquist HJ, Jonsson B, Jónasson PM. Shape polymorphism in Arctic charr, *Salvelinus alpinus*, in Thingvallavatn, Iceland. Physiolg Ecol Jpn. 1989;1:393–404.
83. Pounder KC, Mitchell JL, Thomson JS, Pottinger TG, Sneddon LU. Physiological and behavioural evaluation of common anaesthesia practices in the rainbow trout. Appl Anim Behav Sci. 2017;199:94–102. https://doi.org/10.1016/j.applanim.2017.10.014.
84. Kimmel CB, Ullmann B, Walker C, Wilson C, Currey M, Phillips PC, et al. Evolution and development of facial bone morphology in threespine sticklebacks. PNAS. 2005;102:5791–6. https://doi.org/10.1073/pnas.0408533102.
85. Adams DC, Rohlf FJ, Slice DE. A field comes of age: geometric morphometrics in the 21st century. Hystrix. 2013;24:7–14.
86. Collyer ML, Adams DC. Phenotypic trajectory analysis: comparison of shape change patterns in evolution and ecology. Hystrix. 2013;24:75–83.
87. Morrissey MB, Liefting M. Variation in reaction norms: statistical considerations and biological interpretation. Evolution (N Y). 2016;70:1944–59.
88. Foote M. Contributions of individual taxa to overall morphological disparity. Paleobiology. 1993;19:403–19.
89. Hadfield JD. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R Package. J Stat Softw. 2010;33:1–22.
90. Nakagawa S, Schielzeth H. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biol Rev. 2010;85:935–56.
91. Nakagawa S, Johnson PCD, Schielzeth H. The coefficient of determination R^2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. J R Soc Interface. 2017;14:20170213.
92. Roff DA. The estimation of genetic correlations from phenotypic correlations : a test of Cheverud' s conjecture. Heredity. 1995;74:481–90.
93. Hansen TF, Pélabon C, Houle D. Heritability is not evolvability. Evol Biol. 2011;38:258–77.
94. Jones AG, Arnold SJ, Bürger R. Stability of the G-matrix in a population experiencing pleiotropic mutation, stabilizing selection, and genetic drift. Evolution (N Y). 2003;57:1747–60.
95. Delahaie B, Charmantier A, Chantepie S, Garant D, Porlier M, Teplitsky C. Conserved G-matrices of morphological and life-history traits among continental and island blue tit populations. Heredity (Edinb). 2017;119:76–87. https://doi.org/10.1038/hdy.2017.15.
96. Haber A, Dworkin I. Disintegrating the fly: a mutational perspective on phenotypic integration and covariation. Evolution (N Y). 2016;71:66–80.
97. Arnold SJ, Bürger R, Hohenlohe PA, Ajie BC, Jones AG. Understanding the evolution and stability of the G-matrix. Evolution (N Y). 2008;62:2451–61.
98. Aguirre JD, Hine E, McGuigan K, Blows MW. Comparing G: multivariate analysis of genetic variation in multiple populations. Heredity (Edinb). 2014;112:21–9. https://doi.org/10.1038/hdy.2013.12.
99. Adams DC, Collyer ML. On the comparison of the strength of morphological integration across morphometric datasets. Evolution (N Y). 2016;70:2623–31.

Publisher's Note

Additional file 1: Additional Tables and Figures

Table S1. Pairwise differences of the ontogenetic trajectories of head shape between cross types. Calculated differences, 95% Upper Confidence Limit , standardized scores and *p*-values are shown for three attributes of the trajectories (path length, angle and shape).

	Path length				**Angle**					**Shape**			
	Δd	UCL**x**	*Z*	*p*	*r*	Angle (°)	UCL	*Z*	*p*	*Δd*	UCL	*Z*	*p*
PL-SB	0.01	0.03	-0.28	0.53	0.99	7.03	9.59	-0.72	0.76	0.14	0.22	-0.34	0.62
PL-F_1	0.02	0.04	0.04	0.47	1.00	4.08	6.58	-1.02	0.86	0.09	0.16	-0.66	0.74
SB-F_1	0.03	0.05	0.40	0.35	1.00	3.79	6.81	-1.02	0.86	0.15	0.22	-0.33	0.63

Table S2. Posterior estimates of the Linear Mixed-effect Model on the age of exogeneous feeding (degree days). cross SB= SBxSB-offspring, cross PL= PLxPL offspring, cross F_1 = hybrids.

	Effect	**Posterior mode**	**95% CrI**
Fixed effects	Intercept (cross PL)	651.7	642.5 - 662.1
	cross SB	5.1	-10.4 - 17.5
	cross F_1	3.3	-9.1 - 15.0
Random effect variance	Family	0.5	0.0 - 146.9
Residuals	cross PL	228.3	154.6 - 377.6
	cross SB	323.1	226.1 - 626.9
	cross F_1	253.5	200.7 - 387.4

Table S3. Latency of the focal individual to start feeding across the three observation trials (log seconds).

	Posterior mode	95% CrI
Trial 1	3.56	2.60 - 4.25
Trial 2	3.32	2.37 - 4.01
Trial 3	3.55	2.51 - 4.21
Cross SBxSB	-0.33	-1.54 - 0.76
Cross F_1	-0.17	-1.27 - 0.68
Trial 2*Cross SBxSB	0.53	-0.53 - 1.70
Trial 3*Cross SBxSB	0.20	-0.66 - 1.55
Trial 2*Cross F_1	0.03	-0.89 - 0.90
Trial 3*Cross F_1	0.08	-0.70 - 1.14

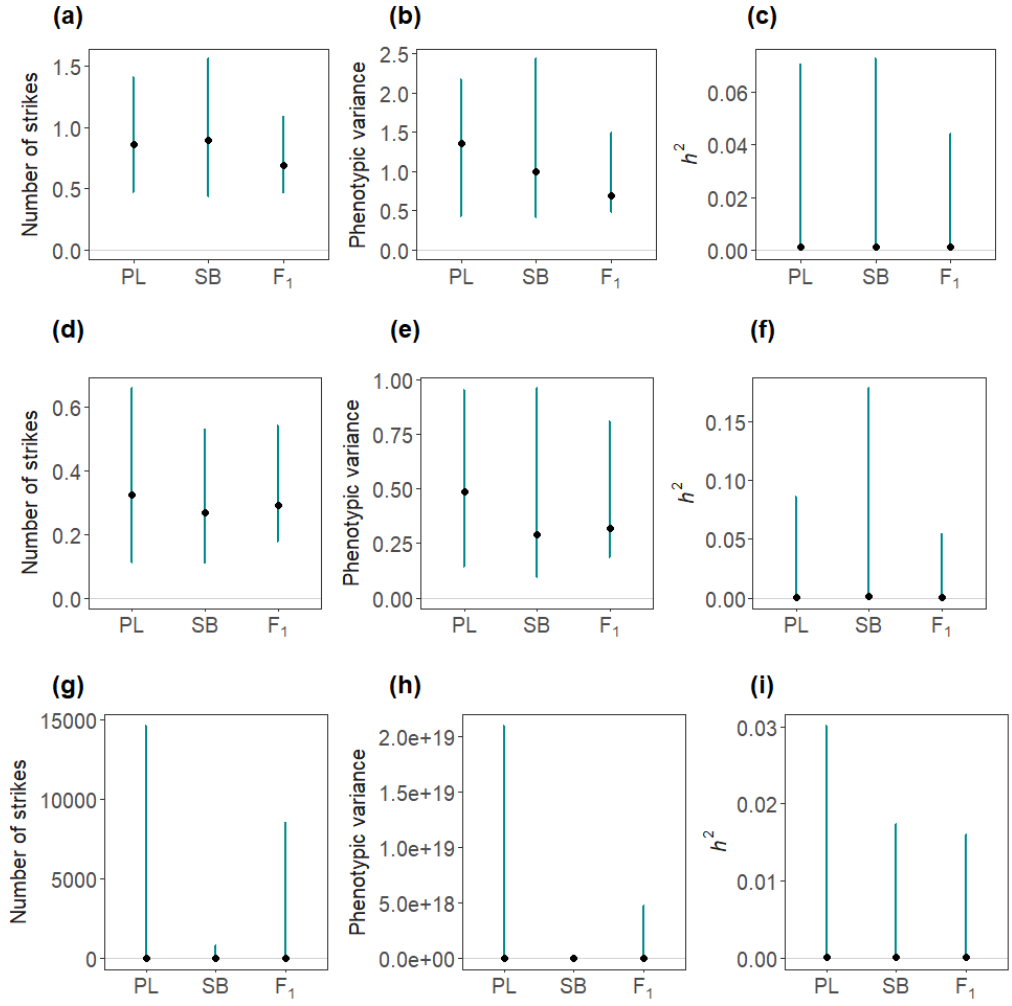

Fig S1. Estimates of feeding behaviours. (a-c) Numbers of feeding attempts on the bottom of the cup, (d-f) at mid-water, (g-i) at the surface. (a-g) Fixed effect estimate, (d-h) total within group variance, (c-i) heritability (h^2). Categories: SB = SBxSB offspring, PL = PLxPL offspring, F_1 = hybrids. Dots: Posterior modes, bars: 95% Credible Intervals.

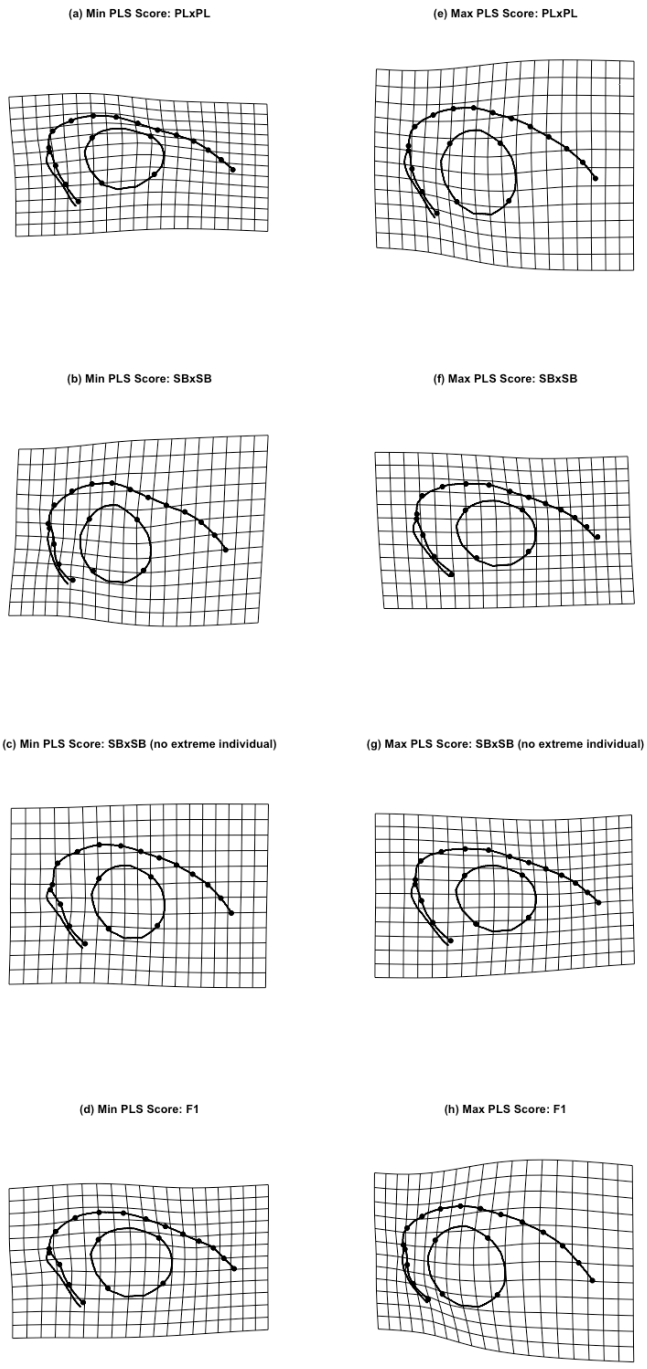

Fig. S2. Thin-plate spline deformation grid depicting shape changes extreme of the PLS axis. (a,e) PLxPL offspring, (b-f) SBxSB offspring, (d-h) F_1 hybrids.

Table S4. Crossing design of the rearing experiment. Cross types: Female gamete x Male gamete. Parents were used for crosses only once (no split families). The numbers of individuals on the left refer to the maximum number individuals at the start of the experiment. The numbers on the right refer to the number of individuals available with no missing data among all the sampling steps. One SBxPL family (19 individuals) hatched two weeks after the others and was not used for the analyses on trait covariance, growth, morphology and feeding behaviour.

Cross type	Number of families	Number of individuals
PLxPL	2	64 - 37
SBxSB	2	42 -15
PLxSB	3	75 - 23
SBxPL	2	49 -18

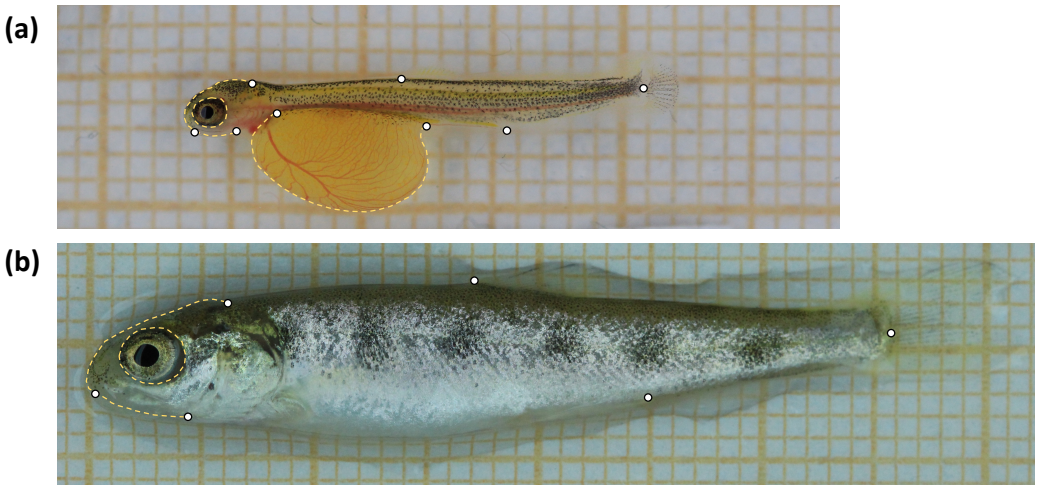

Fig. S3 Landmarks used for the analyses of shape differences. (a) On free-swimming embryos at hatching (D1: ca. 445 °C *d*ays) and (b) on actively feeding juveniles (D4: ca. 1100 °C *d*ays). The dashed curves depict the location of the Bezier curves used to extract the semi-landmarks. The specimens presented here are from the PLxPL offspring.

Table S5. Specifications of the Generalized linear mixed-effect models run the analyses to the separate traits. For all model, burnin = 300 x number of iterations, thinning intervals = 10 x number of iterations.

Trait	Fixed effects	Random variable	Model specificities	Number of iterations
Growth				
Log standard length (average differences)	Cross type x Age	Family Individual	Random regression Second order polynomial regression	$6.5x10^4$
Log standard length (within-group variation)	Age + Family	Individual	One model per type of cross	$1.3x10^6$
Yolk sac size at hatching and resorption				
Yolk sac area at hatching, Yolk sac area at hatching + 20 days (average differences)	Standard length + Cross type	Family Individual	Multi-response model	$3.9x10^6$
Yolk sac area at hatching, Yolk sac area at hatching + 20 days (within-group variation)	Standard length Family	Individual	Multi-response model One model per type of cross	$4.6\ x10^6$
Feeding behaviour				
Age of exogeneous feeding	Cross type	Family		$2.6\ x10^5$
Propensity to feed	Feeding trial x Cross type	Family Individual	Binomial GLMM with logit link	$3.9\ x10^6$
Log Latency to feed	Feeding trial x Cross type	Family Individual		$2.6\ x10^5$
Total Number of feeding attempts	Feeding trial x Cross type	Family Individual	GLMM with log link	$5.7\ x10^7$

Number of feeding attempts (bottom)	Total Number of feeding attempts + Cross type	Family Individual	GLMM with log link	1.0 x10^7
Number of feeding attempts (water column)	Total Number of feeding attempts + Cross type	Family Individual	GLMM with log link	1.0 x10^7
Number of feeding attempts (surface)	Total Number of feeding attempts + Cross type	Family Individual	GLMM with log link	1.0 x10^7

Table S6. List of the individuals that were discarded from the analyses.

	Type of cross	Explanation
Removed from the morphological analyses at hatching	**PLxPL**	**Identified as outlier in MANOVAs on body shape at hatching. Malformed mandibula and odd yolk sac shape on the photographs.**
	SBxSB	**Heavily malformed craniofacial morphology ("bulldog" face)**
	SBxSB	**Heavily malformed craniofacial morphology ("bulldog" face)**
Removed from the behavioural analyses	**PLxSB**	**Individual unable to adjust its buoyancy**
	SBxPL	**Twisted spine constraining swimming activities**
	PLxSB	**Head infected by fungi**
	SBxSB	**Twisted spine constraining swimming activities**
	SBxSB	**Individual unable to adjust its buoyancy**
	PLxPL	**Yolk sac not depleted and containing air**

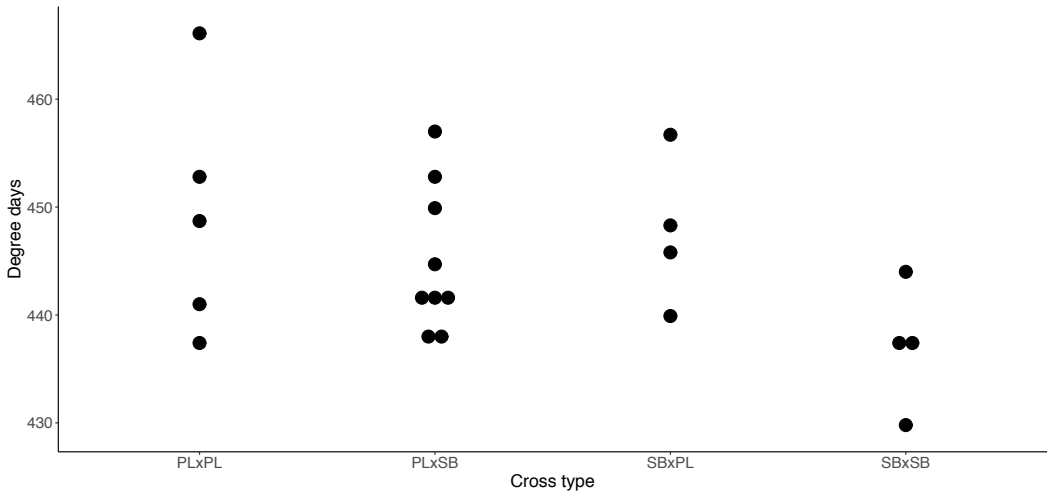

Fig. S4 Developmental time points of the successfully fertilised families at hatching.

Paper II

Animal Behaviour 175 (2021) 57–73

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Animal personality adds complexity to the processes of divergence between sympatric morphs of Arctic charr

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ARTICLE INFO

Article history:
Received 11 June 2020
Initial acceptance 20 August 2020
Final acceptance 1 February 2021

MS number 20-00431R

Keywords:
adaptive divergence
animal personality
behavioural syndrome
reproductive isolation
resource polymorphism
speciation

Divergent selection is a powerful driver of speciation and has been widely studied in relation to the physical characters of organisms. Behavioural traits also significantly contribute to the evolutionary processes of divergence. However, studying such effects is fraught with difficulties as the development of behavioural traits is likely to be complex and is moulded by ontogenetic processes such as shifts in habitat use. Here we explored how several aspects of juvenile behavioural variation may relate to adaptive divergence in a freshwater fish. We assessed whether and how juveniles of two recently evolved, sympatric morphs of Arctic charr, *Salvelinus alpinus*, a small benthivorous and a planktivorous charr, show genetically based differences in personality that conform to their respective ecological niches, and whether these differences could contribute to reproductive isolation by generating maladaptive hybrid behaviours. Studying three aspects of behavioural variation (average trait value, consistent individual differences and trait correlations), we assessed the sociality and risk-taking propensity of hybrids and pure-morph offspring reared in common conditions. While no difference in average behavioural responses could be observed, the hybrids tended to show less repeatable behaviours and were not intermediate for behavioural syndromes that appear to differ between the two morphs. These results provide limited evidence of personality trait divergence among polymorphic fish, and suggest subtle, nonadditive effects of hybridization on the development of such traits.

A variety of behavioural traits are now widely recognized as playing a key role in the evolution of animal populations through natural selection (Wilson, 1998; Wolf & Weissing, 2012). Traits such as aggressivity, sociality and boldness (the propensity for taking risks) are well studied for their implications for foraging success, predator avoidance, vulnerability to parasites, breeding success and life history strategies in a wide range of taxa (Réale et al., 2010; Sinn, Apiolaza, & Moltschaniwskyj, 2006; Smith & Blumstein, 2008). Major advances in our understanding of the role of behaviour in evolution have been recently achieved by focusing on individual differences that remain consistent over time and contexts, that is, animal personality (Roche, Careau, & Binning, 2016). Behavioural differences between populations are now recognized to be more than differences in mean trait values and can be studied under different aspects, such as (1) the group level, average values of a given behavioural trait, (2) the consistent differences between individuals in this trait (personality per se) and (3) the correlations between this trait and others, here defined as behavioural syndromes (Sih, Cote, Evans, Fogarty, & Pruitt, 2012). Because of their genetic bases and their effects on fitness, personality and behavioural syndromes are considered to be important drivers of adaptive divergence and speciation (Ingley & Johnson, 2014; Johnson, Culumber, Easterling, & Rosenthal, 2015; Wolf & Weissing, 2012). Considering classical models of speciation (Nosil, 2012), divergent selection may indeed result in the segregation of behavioural types between different fitness optima. Reproductive isolation, such as the selection against hybrids with phenotypes that are intermediate or outside the range of parental values (i.e. transgressive phenotypes), could then develop as a by-product, which may in turn lead to further divergence (Schluter, 2001). Despite the recent

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https://doi.org/10.1016/j.anbehav.2021.02.022
0003-3472/

conceptual advances on the importance of personality in the processes of adaptive divergence and speciation, empirical studies directly investigating it are still lacking (Ingley & Johnson, 2014). Information is especially lacking regarding the behavioural phenotype of hybrids and their contribution to reproductive isolation (Rice & McQuillan, 2018).

Here we explore whether behaviour as considered under the three aspects described above (average trait value, consistent differences between individuals and trait correlations) can be involved in the evolutionary processes of divergence and speciation. First, contrasting ecological conditions can generate different fitness optima that favour the differentiation of populations in the average values of a behavioural response (i.e. 'behavioural adjustment', Fig. A1a; Barbosa et al., 2018). Second, contrasting environmental variables such as the predictability of a food resource or predation risk can determine the benefit of behavioural consistency over plasticity, thus affecting the level of consistent differences between individuals (i.e. personality per se; Dall, Houston, & McNamara, 2004), a process defined as behavioural 'homogenization' versus 'diversification' in Barbosa et al. (2018; Fig. A1b,c). Finally, because of genetic constraints, functional trade-offs and correlational selection (Arnold, 1992), correlations between traits can be important determinants of fitness and one may therefore expect personality syndromes to be shaped differently between diverging populations (Dingemanse et al., 2007). These three aspects of behavioural variation could therefore affect the build-up of reproductive isolation if hybrids present either (1) disadvantageous average values in personality traits, (2) a loss in behavioural consistency (personality breakdown) or (3) maladaptive combinations of these traits (syndrome breakdown).

Postglacial lakes hosting different varieties or morphs of freshwater fish are particularly valuable biological systems offering a glimpse of early stages of divergence (Skúlason et al., 2019). These lakes often contain sympatric populations, which facilitate the study of divergent selection by limiting the effects of geographical barriers on gene flow. The evolution of these systems has been described under the framework of resource polymorphism, where a few fish species colonized recently deglaciated lakes offering a variety of unoccupied ecological niches, thus promoting the emergence of different sympatric morphs. These morphs usually segregate between the benthic and the limnetic habitats and are characterized by various levels of reproductive isolation (Skúlason et al., 2019; Snorrason & Skúlason, 2004). The Arctic charr, *Salvelinus alpinus*, from the Icelandic lake Thingvallavatn presents an extreme and rapid case of such divergence that resulted in the emergence of four lake-locked morphs which are evolving (at least in their current state) in sympatry (Fig. 1).

We focus here on two of the four morphs in Thingvallavatn, the 'small-benthic' (SB) and the 'planktivorous' (PL) charrs. The SB charr live in the stony littoral zone of the lake and forage on benthic invertebrates, mainly the snail *Radix peregra* and chironomid larvae. In this habitat they use their small size to manoeuvre among the lava stones to access food and seek shelter from predation. The PL charr utilize the pelagic zone of the lake and feed on zooplankton and emerging chironomid pupae. The spawning seasons of SB and PL charr overlap (the spawning season of SB charr encompassing that of PL charr) and these two morphs appear to also share their spawning locations (Skúlason, Snorrason, Noakes, Ferguson, & Malmquist, 1989). Estimates of gene flow between the two charr are however very low and individuals of intermediate morphology are rarely observed (Guðbrandsson et al., 2019; Kapralova et al., 2011) in spite of the ease of generating mature first-generation hybrids (F_1) in captivity. These observations suggest that selection against hybrids may, to some extent, contribute to the reproductive isolation of the two morphs.

The young of the year of the two morphs are believed to use the same habitat, the surf zone (0–1 m deep), from the onset of active feeding in spring until the PL charr start shifting towards deeper pelagic and epibenthic zones around mid-summer (Sandlund et al., 1988). Few data on the ecology of the juveniles of PL and SB charr are, however, available, and the exact timing and synchronicity of the ontogenetic niche shift of young of the year PL charr and its behavioural consequences are unknown. The laboratory-reared offspring of the two morphs nevertheless differ in their morphology and in their patterns of gene expression before this presupposed shift (Guðbrandsson et al., 2018; Kapralova, 2014; Kapralova et al., 2015), and develop sharp genetically based differences in foraging behaviour within the year of hatching (Skúlason, Snorrason, Ota, & Noakes, 1993). Given these observations and considering that differences in personality traits can extend over ontogenetic stages (Herczeg, Ab Ghani, & Merilä, 2013; Polverino, Santostefano, Díaz-Gil, & Mehner, 2018) and can be genetically correlated with morphological variation (Kern, Robinson, Gass, Godwin, & Langerhans, 2016), we hypothesized that the young of the year offspring of the two morphs could exhibit differences in personality traits already reflecting the extensive ecological differences observed at the adult stage.

Based on the ecological characteristics of the two morphs described above, we expected them to have evolved differences in boldness and sociality, two personality traits known to be often related to fitness (Réale et al., 2010; Smith & Blumstein, 2008). These differences should be observed as changes in the three behavioural aspects discussed above. Briefly, we expected the PL charr to have evolved personality traits favouring the formation of shoals. Shoaling formation is known for being advantageous in open habitats with low physical complexity (Orpwood, Magurran, Armstrong, & Griffiths, 2008). During the summer and autumn, juvenile and adult PL charr forage in open water environments where they spread out during the dusk and dark hours, but form dense shoals or stay deeper at full daylight; probably as a predator avoidance tactic (Jónasson, 1992). Considering that social and territorial behaviours were found to have genetic bases in related species of *Salvelinus* (Ferguson & Noakes, 1982), we predicted that PL charr would display higher average values in social behaviours than SB charr. We also predicted reduced between-individual differences in PL charr as selection would have depleted the genetic variation related to these traits. Moreover, because bold individuals

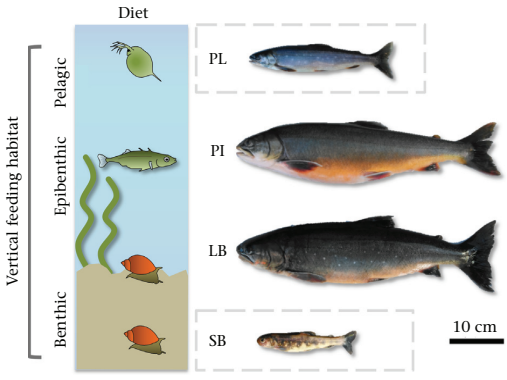


Figure 1. The four morphs of Thingvallavatn with their foraging habitat and main prey depicted on the left (from top to bottom: planktonic crustacean, fish, freshwater snail). All four specimens were captured together on the same site. PL: planktivorous; PI: piscivorous; LB: large benthic; SB: small benthic charr. The two focal morphs of our study (PL and SB) are highlighted with dashed lines.

appear to have lower propensities for social behaviours (Ward, Thomas, Hart, & Krause, 2004), we expected PL charr to have reduced average values as well as reduced between-individual differences in boldness as a response to selection for social personality types. In contrast, the high physical complexity of the habitats occupied by SB charr may not only relax the selection on social traits but also favour the establishment of a high diversity of personality types, for example by enabling bolder SB charr that are more likely to move across foraging areas or feed away from shelters to thrive with shyer individuals that tend to stay within sheltered areas (e.g. fissures and restricted spaces between boulders).

We raised individuals from pure-morph and hybrid crosses in common garden conditions to characterize the range of behavioural differences between the types of crosses regarding the three different aspects of variation. We expected the importance of the boldness and sociality traits in this case of divergence to be revealed by genetically based differences between SB and PL individuals according to the predictions described above. Moreover, because the merging of two diverging genomes often produces either maladaptive intermediate or transgressive hybrid traits (Albertson & Kocher, 2005), genetically based behaviour variations in hybrids falling outside the range of the two morphs should reveal whether they would be selected against.

METHODS

Study System

Thingvallavatn is Iceland's largest lake, with an area of 84 km^2 and a mean depth of 34 m. The lake sits in a graben of the Mid-Atlantic ridge and was formed following the last glacial retreat about 10 000 years ago (Pétursson, Norðdahl, & Ingólfsson, 2015). The physical structure of the lake is characterized by a wide pelagic zone and three major benthic habitats: a 'stony littoral' zone (0–10 m deep) composed of a spatially complex lava substrate with loose stones, crevasses and interstitial spaces, a densely vegetated zone of *Nitella opaca* algae (10–20 m deep) and a profundal zone (25 m and deeper) where the bottom is covered by a diatomic gyttja substrate (Sandlund et al., 1992). The four morphs of Arctic charr (the planktivorous, the piscivorous, the large-benthic and the small-benthic) differ in habitat use, diet, head and body morphology, life history and parasitism (Sandlund et al., 1992), and constitute at least three genetically differentiated populations (the status of the piscivorous charr remains unresolved; Guðbrandsson et al., 2019). All four morphs are completely sympatric, although coalescent models are consistent with scenarios involving short periods of geographical isolation between PL and SB charr (Kapralova et al., 2011). The two morphs of our study overlap in their spawning seasons (SB: August–November; PL: September–November) but show genetically based differences in head shape, growth patterns and foraging strategies (Skúlason et al., 1993; Skúlason, Noakes, & Snorrason, 1989; Snorrason et al., 1994).

Field Sampling of Parental Specimens and Offspring Rearing

We collected adult specimens in October 2017 by laying gillnets overnight on a spawning site used by the two morphs (Svínanesvík, 64°11′24.6″N; 21°05′40.5″W). We crossed the gametes of 18 ripe specimens as soon as they were brought ashore to generate nine full-sibling families of pure-morph (Female x Male parents: PLxPL and SBxSB) and hybrid crosses (PLxSB and SBxPL, see the crossing design in Table 1). The eggs were incubated in a single EWOS hatching tray (EWOS, Norway) at 4.1 ± 0.2 °C in the aquaculture facilities of Hólar University, Sauðárkrókur, Iceland. Hatching occurred in January 2018 and 20–40 free-swimming embryos per family were moved to single-individual cells in a common water flow-through tank on their hatching day (when 50% of the clutch was hatched). Soon before the onset of active feeding (ca. 530 degree days, March 2018), we replaced the cells by 22 cl identifiable, perforated and transparent cups allowing the exchange of olfactory cues as well as visual contact between individuals. At the same time, groups of ca. 20 fish that were not selected for the rearing experiment were moved into family-specific containers. These fish were used as test shoals for the experiment on sociality. All the fish were fed daily ad libitum with aquaculture pellets. To homogenize the potential environmental effects on personality (e.g. social environment, water flow and temperature variations) among types of crosses, the locations of the individual cells within the set-up were shuffled once per week.

Behavioural Experiments

We conducted two types of experimental tests during a 5-month period after hatching, that is, 2 months after the onset of exogenous feeding (May 2018, ca. 1100 degree days). In the wild this corresponds to the period when the juveniles of both morphs stay in the littoral zone, shortly before PL charr shift towards deeper habitats (Sandlund et al., 1992). We expected behavioural differences to occur at this point as the juveniles of both morphs have already developed different morphologies of their feeding apparatus (Kapralova, 2014; Kapralova et al., 2015), and because differences in Arctic charr foraging behaviours emerge in the first few weeks following the onset of exogeneous feeding (Leblanc, Benhaïm, Hansen, Kristjánsson, & Skúlason, 2011). We therefore terminated the experiment at this time point to minimize the amount of time spent by the fish inside the individual containers.

The first test was aimed at assessing the position of each fish along boldness/shyness axis ('boldness test'). The second test quantified the sociality of the same individuals ('sociality test'). Both tests relied on the video tracking of a focal individual using the software Ethovision XT 8.5 (Noldus Information Technology, Wageningen, The Netherlands). We used 93 fish reared in the cups as focal individuals (37 PLxPL charr, 15 SBxSB charr, 23 and 18 F_1 hybrids of PLxSB and SBxPL maternal origin, respectively; Table 1).

The boldness test consisted of an open-field test with shelter. These tests are commonly used to assess boldness in fish through measurements of activity (distance moved), thigmotaxis (the aversion for locations away from vertical surfaces) and/or the time taken to exit a shelter (Benhaïm, Ferrari, Chatain, & Bégout, 2016; Dahlbom, Lagman, Lundstedt-Enkel, Sundström, & Winberg, 2011; Kern et al., 2016). The set-up was composed of a 40 × 30 cm and 25 cm high arena which was filled with 10 litres of water from the tap of the rearing tray (Fig. A2). The bottom left corner of each compartment contained an opaque white PVC shelter box (11 × 6.5 cm and 6 cm high) closed by a vertical sliding trapdoor. The area was divided into four virtual zones in relation to the shelter, the entrance zone of the shelter, a marginal zone and a central zone of the arena deemed to be the area of high risk. The

Table 1
Crossing design of the rearing experiment and number of individuals used for the personality tests

Type of cross[a]	Number of families	Number of individuals
PLxPL	2	37
SBxSB	2	15
PLxSB	3	23
SBxPL	2	18

[a] Type of cross: Female gamete x Male gamete. The parents were used only once (no half-siblings).

width of the marginal zone was defined as twice the body length of the focal fish. The test started by introducing the focal fish into the shelter from the upper side through a 2 cm wide aperture, immediately sealed with a lid after the introduction. The trap door was gently opened after a 5 min acclimatization period and a 20 min video-recording trial was simultaneously initiated. Twelve behavioural variables were extracted from the video output (Fig. 2, Table A1). The test was carried out twice for every individual with a 7-day interval between trials to capture the behavioural variation related to both within- and between-individual differences. At the end of the first trial, the fish was lightly anaesthetized with 2-phenoxyethanol (see the Ethical Note) and a lateral view photograph of the left side of the specimen was taken for morphometric purposes using a down-facing fixed camera (Canon EOS 650D with a 100 mm macro lens). The fish was then returned to the rearing tray.

The sociality tests were started 1 week after the last boldness trial, for which we used an 80 × 30 cm and 15 cm high arena divided into three compartments (Fig. A2). A central compartment (10 × 30 cm) contained the focal fish and was separated from two side compartments (10 × 30 cm) by transparent acrylic walls. These walls were perforated to allow transfer of chemical cues while preventing the fish from moving between compartments. A 'start box' made of a vertical cylinder (10.5 cm high × 10.5 cm inner-side diameter) was placed in the middle of the arena. Five fish of the same type of cross as the focal individual and raised in a group since hatching were placed together in one of the two side compartments. The focal fish was introduced into the start box through a 2 cm diameter door on the upper side. The start box was removed after an acclimatization period of 5 min and a 20 min videorecord was initiated.

As with the experiment on boldness, two replicate trials were conducted for each fish, 1 week apart, but the side compartment containing the group of congeners was alternated between the two rounds. The water of the arenas was always renewed between observations to mitigate the presence of chemical cues from the previous focal fish as well as temperature changes. To minimize stress, the fish were transported to the experimental room inside covered buckets filled with the same water as the common garden set-up. The trials were recorded using a video camera (IMAGING SOURCE DMK 21AU04, 640×480 pixels) placed 180 cm above the centre of the multiarena set-ups and operated with the software IC-capture 2.4 (The Imagine Source, 2014) with a frame rate of 30 Hz. After the last trial, each fish was euthanized with an overdose of 2-phenoxyethanol (Pounder, Mitchell, Thomson, Pottinger, & Sneddon, 2018), and measured for wet body mass and fork length.

Statistical Analyses

Boldness can be seen as a complex, integrated trait involving multiple behaviours (Goodchild, Schmidt, & Durant, 2020; Réale, Reader, Sol, McDougall, & Dingemanse, 2007). We therefore developed a straightforward boldness/shyness index based on the 12 variables recorded during the open-field tests by conducting an exploratory factor analysis (Fig. 2, Table A2). Briefly, this method characterizes unobserved 'latent' variables associated with sets of correlated observed variables (Bollen, 2002), in our case using factor rotations. Principal component analyses (PCA) are also commonly used to derive personality scores (Church & Grant, 2018; Kern et al., 2016) but have the disadvantage of constraining the variables to orthogonality (i.e. the new axes cannot be correlated) and of sometimes inflating the factor loadings (Budaev, 2010). We, however, conducted a PCA on the same data set as a simple complementary method to the factor analysis to visually explore the most important patterns of covariation among the observed variables.

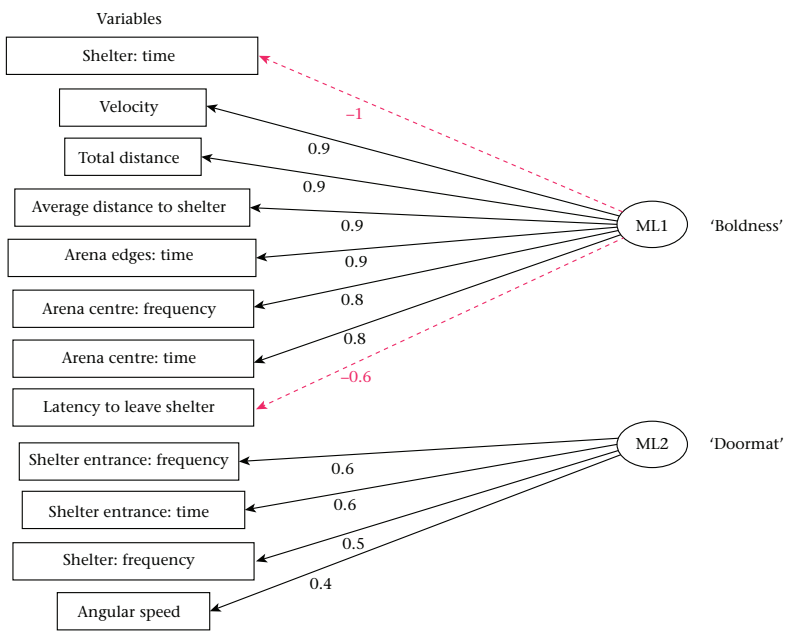


Figure 2. The two latent variables (ML1 and ML2) generated through factor analyses of the variables measured during the boldness test (boxes). Numbers on the arrows: factor loadings. Continuous lines: positive correlation; dashed lines: negative correlations. ML1 and ML2 are referred to in the subsequent analyses as 'boldness' and the 'doormat' trait, respectively. The detailed description of observed variables is in Table A1.

We ran the factor analysis using the maximum likelihood method and Oblimin rotations available in the R package psych (Revelle, 2019), and identified two latent variables (Fig. 2). One latent variable was related to observed variables describing a classical 'boldness/shyness' axis as well as exploratory tendencies (e.g. travelled distance, velocity), and will hereafter be referred to as the boldness trait. The second latent variable regrouped the observed variables related to the use of the entrance of the shelter (e.g. entry and exit frequencies, time spent in the entrance zone) and the angular speed. This variable was distinct from the characteristic axes of boldness and exploration, and also differed from the other classical categories of personality trait (Réale et al., 2007). Because this latent variable mostly described the intensity at which the area near the entrance of the shelter was used, we named it a doormat trait. Note that the visual inspections of the first two principal components of the PCA also revealed two groups of observed variables that reflected the boldness and doormat traits (Fig. A3).

Sociality was studied in a more straightforward way than boldness because the test used here limited the possibility of social interactions between the focal fish and the individuals from the shoal, thereby limiting the variety of variables to collect. We therefore studied sociality as a univariate variable using the average distance between the focal fish and the compartment containing the congeners.

To account for the confounding effect the physical condition of the fish may have on their behavioural response, we extracted individual indexes of body condition as residuals from a regression of the wet weight of the specimen over its standard length (García-Berthou, 2001; Fig. A4, Table A2). This value was extracted from the morphometric photographs using the R packages StereoMorph and Geomoporph (Adams & Otárola-Castillo, 2013; Olsen & Westneat, 2015).

We used multiresponse linear mixed models (Hadfield, 2010) to test whether the types of cross differed in the three behavioural aspects ((1) average trait value, (2) consistent individual differences and (3) trait correlations). The values of the three traits (boldness, doormat, sociality), mean centred and scaled by their respective standard deviations, were used as a multiple response. We studied behavioural aspect 1 by assessing the importance of the type of cross as a fixed effect in a multiresponse model containing standard length, body condition and trial number as covariates. The identity of the specimen and its family were added as random variables. We assessed the importance of the effect of the type of cross relative to the total amount of behavioural variation by adapting the marginalized determination coefficient (R^2_m) from Nakagawa and Schielzeth (2013):

$$R^2_{(m)} = \frac{V_{cross}}{V_{fix} + V_{ind} + V_{fam} + V_e} \quad (1)$$

where V_{cross} and V_{fix} are the variances calculated from the fixed-effect component referring to the type of cross alone and to all fixed effects, respectively. V_{ind} and V_{fam} are the variance components associated with the differences between the intercept of individuals and of families, respectively, and V_e is the residual variance (within-individual variance).

The differences between types of crosses related to the second and the third behavioural aspects (consistent individual differences and behaviour syndrome) were assessed by extracting the variance and covariance components of three separate models (one per type of cross). These models contained the standard length, body condition, trial number and family identity as fixed effects while the individual identity was set as a random variable. From these three models, we assessed the amount of consistent differences between individuals in each trait and in each type of cross by calculating their adjusted repeatability (R). The adjusted repeatability controls for confounding factors (here body condition, size, trial number and family) and was calculated using the formulation from Nakagawa and Schielzeth (2010) and Villemereuil, Morrissey, Nakagawa, and Schielzeth (2018):

$$R = \frac{V_{ind}}{(V_{ind} + V_e)} \quad (2)$$

Finally, we tested for differences in correlations between traits (an important component of behavioural syndromes, aspect 3) among types of cross by comparing the between-trait correlation coefficients extracted from the variance–covariance matrices of the three models. To gain statistical power, the two categories of reciprocal hybrids were pooled for all models.

We fitted all the models under a Bayesian framework using Markov chain Monte Carlo (MCMC) methods as implemented in the package MCMCglmm (Hadfield, 2010). This approach is especially suitable for analyses with constrained sample sizes that are inherent to the studies of wild, nonmodel organisms (Garamszegi, 2016). We specified weakly informative priors ($V_{0family} = 1$, V_{0ind} and V_{0res} = identity matrix I_3 , nu = 3) and determined the number of iterations allowing model convergence through the examination of trace plots, posterior density plots and effective sample sizes. The final number of MCMC iterations, thinning interval and burn-in were 1.3×10^5, 1×10^4 and 3×10^4, respectively, for the model on the average trait values (Model 1), and were 3.9×10^6, 3×10^5 and 9×10^5 for the three separate models on repeatability and trait correlations. Because analyses on individual correlations may lack power in studies with two measurements per individuals (Dingemanse & Dochtermann, 2012), and because there is no clear-cut rule for interpreting Bayesian probabilities, inferences were made by comparing altogether the posterior modes, the 95% credible intervals (CrI) and the posterior densities of the estimated values among the types of crosses. The underlying R codes and data sets are provided in the Supplementary Material.

Ethical Note

Sampling was conducted with the permissions of the owner of the farm of Mjóanes and the Thingvellir National Park commission. Ethics committee approvals for the research project were not required by Icelandic regulation (Act No. 55/2013 on Animal Welfare). The rearing and the experimental work were, however, conducted in the facilities of Hólar University Aquaculture Research Station, which has an operational licence according to the Icelandic Aquaculture law (Law No. 71/2018). This law includes best practices for animal care and experimental work. All the fish were killed according to the most careful euthanasia practices for salmonid fish (Pounder et al., 2018) and the optimal dosage for anaesthesia with 2-phenoxyethanol was adjusted to the reactions of the individual, following the recommendations of the laboratory facility. Decisions on the sample size and on the design of the common garden experiment were made to ensure that a companion study and other projects on hybrid charr development could be conducted with the data collected on the same specimens.

RESULTS

Differences in Average Trait Values

We found that the boldness scores tended to be lower and the average distances to conspecifics tended to be higher (lower sociality) in SBxSB offspring and hybrids than in PLxPL offspring

(Table 2). The effect of the type of cross, however, explained a small proportion of the total variation ($R^2_{(m)}$: posterior mode [95% CrI] = 0.04 [0.01; 0.11]). These results were also observed as limited trends in the graphical representations of the reaction norms of each trait (Fig. A5) but were also nonsignificant when employing separate linear mixed models with a single, nonscaled trait as a response (Table A4).

Consistent Individual Differences

The repeatability estimates were high in the PLxPL and in the SBxSB offspring for boldness (PLxPL: $R = 0.58$ [0.36; 0.74]; SBxSB: $R = 0.68$ [0.37; 0.86], posterior mode [95% CrI]) and the doormat behaviour (PLxPL: $R = 0.55$ [0.32; 0.72]; SBxSB: $R = 0.55$ [0.28; 0.80]). Although the repeatability of boldness appeared slightly lower in the PLxPL offspring compared to the SBxSB offspring, wide overlaps among their respective 95% CrIs did not provide a high level of certainty regarding these differences. These results indicate extensive consistent individual differences in these two traits for both morphs (Fig. 3a). Repeatability estimates were also different from zero in the offspring from pure-morph crosses for the sociality trait and did not appear to differ between the two groups (PLxPL: $R = 0.38$ [0.21; 0.57]; SBxSB: $R = 0.43$ [0.21; 0.71]).

The hybrids also showed high repeatability in the three traits (boldness: $R = 0.46$ [0.26: 0.62]; doormat: $R = 0.36$ [0.20; 0.52]; sociality: $R = 0.45$ [0.22; 0.59]). The posterior modes of boldness and the doormat behaviours were, however, lower in the hybrids than in the offspring from pure-morph crosses, and the overlaps in 95% CrI provide a moderate level of certainty regarding reduced repeatability in the hybrids for these two traits (Fig. 3a). Focusing on the repeatability components (i.e. the between- and within-individual variances), we observed that a relatively higher within-individual variance in the hybrids for these two traits may explain their reduced repeatability (Fig. 3b and c).

Behavioural Syndromes

We also observed differences in the posterior estimates of trait correlations (i.e. behavioural syndromes) between the types of crosses (Fig. 4a and b, Table A5). Among the SBxSB offspring, the posterior modes showed a positive correlation between the boldness and the doormat behaviour ($\rho = 0.42$ [-0.23,0.78], posterior mode [95% CrI]). This indicated that the bolder the SBXSB charr were, the more intensively they displayed behaviours related to use of the entrance of the shelter. The same trait correlation, however, tended to be negative in the PLxPL offspring ($\rho = -0.17$ [-0.57,0.21]). Furthermore, the pairwise comparison of the correlation estimates suggested that the offspring of the two types of pure-morph crosses differed in a syndrome involving the boldness and the doormat traits, although overlapping 95% CrIs conferred a limited statistical support to this trend (Fig. 4b). The doormat behaviour and sociality did not appear to be correlated in either of the pure-morph offspring (PLxPL: $\rho = 0.0$ [-0.50; 0.38]; SBxSB: $\rho = -0.017$ [-0.75; 0.45]), and these types of crosses did not differ from one another regarding this aspect of behavioural variation (Fig. 4b). The PLxPL offspring, however, tended to show a positive correlation between the boldness scores and sociality (bolder PLxPL charr tended to be more social, $\rho = 0.19$ [-0.26; 0.60]) while this correlation appeared to be null in the SBxSB offspring ($\rho = 0.00$ [-0.66; 0.50]). The analysis of the pairwise differences in posterior estimates, however, only provided a weak support for differences between the two types of crosses in this trait correlation (Fig. 4b).

Differences were also observed in the trait correlations in hybrids compared to those of the offspring from pure-morph crosses (Fig. 4, Table A5). The correlation between boldness and doormat appeared to be negative in the hybrids ($\rho = -0.40$ [-0.65; 0.14], posterior mode [95% CrI]), indicating that bolder hybrid charr display less behaviour related to the use of the shelter entrance. The hybrids differed from the SBxSB offspring (which showed the opposite correlation) but not from the PLxPL offspring (Fig. 4b). However, the correlation between sociality and doormat was negative in the hybrids ($\rho = -0.31$ [-0.68; 0.10]) and tended to differ from the PLxPL charr (Fig. 4b). A positive correlation between boldness and sociality was also observed in the hybrids ($\rho = 0.29$ [-0.19; 0.63]), but this relationship did not appear to differ from the ones observed in the offspring from the two types of pure-morph crosses (Table 2).

Table 2
Posterior modes and 95% credible intervals (CrI) of the estimates of each trait and for each type of cross

Trait	Cross type	Posterior mode	95% CrI		
Boldness	SBxSB	-0.60	-2.32	–	0.66
	F_1 Hybrids	-0.76	-1.69	–	0.77
Doormat	SBxSB	-0.49	-1.89	–	1.13
	F_1 Hybrids	0.19	-1.13	–	1.40
Sociality	SBxSB	0.64	-1.01	–	1.90
	F_1 Hybrids	0.49	-0.78	–	1.65

For each trait, the pure-morph cross PLxPL constitutes the baseline (0). Boldness and doormat are scores from latent variables; sociality is quantified as the average distance to conspecific (cm). The three variables are mean-centred and scaled by their standard deviation. More details on the posterior estimates and posterior distributions of this model can be found in Table A3 and Fig. A6.

DISCUSSION

Like any trait with genetic bases, behavioural traits undergoing adaptive divergence can be revealed through common garden experiments (Herczeg et al., 2013). Our data provide little support to our initial hypothesis that adaptive divergence might have acted on the three aspects of behavioural variation studied here in such a way that differences could be observed after the fish had started feeding actively and before moving into the contrasting adult habitats. We did not observe differences between morphs in average trait values at this early stage. Furthermore, the offspring of the two morphs showed similar patterns of repeatability for all three traits. Note that the repeatability estimates for all traits were within the range of values observed in teleost fish, including salmonids, although the boldness estimates we observed were relatively high (Bell, Hankison, & Laskowski, 2009; Church & Grant, 2018). However, the data indicated some contrasts in behavioural syndromes between the two morphs as seen mostly through the opposite signs of the correlations between boldness and doormat.

The hybrids differed from the two morphs in a complex way. Compared to the offspring of the pure-morph crosses the hybrids showed a trend towards reduced repeatability in the boldness and doormat traits. Just like the pure PLxPL offspring, the hybrids showed a negative correlation of boldness and doormat, opposite to what was seen in SBxSB. Note that there are no straightforward policies about multiple testing using Bayesian approaches (Berry & Hochberg, 1999; Sjölander & Vansteelandt, 2019). However, in light of the number of behavioural aspects tested in this study, indications of differences in trait correlation warrant interpretation. Regarding our other results, the lack of observed differences and the trends with limited statistical support also need to be interpreted considering the limited sample size of this study, especially in view of the modest number of families per cross type (two for each pure-morph cross and three for the hybrid crosses).

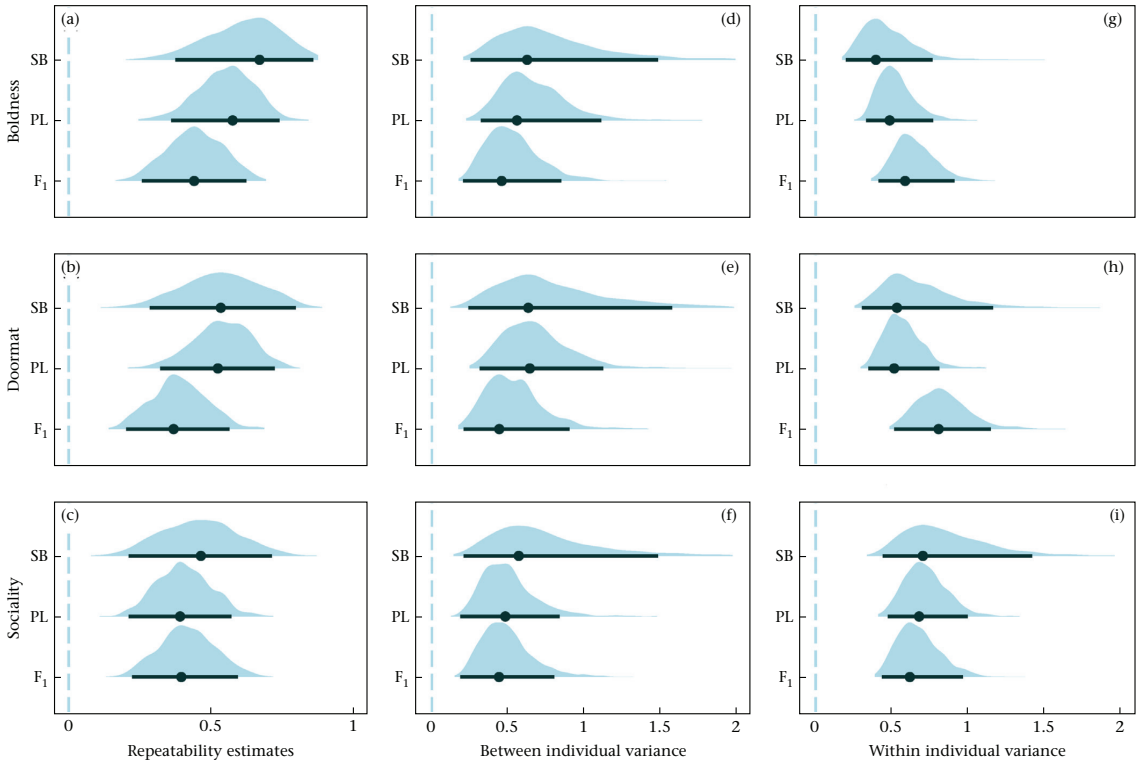


Figure 3. Posterior distributions (blue shading), posterior mode (circle) and 95% credible intervals (horizontal line) of the estimates of (a, b, c) repeatability, (d, e, f) between-individual variance and (g, h, i) within-individual variance of each trait in the three types of crosses (SB: SBxSB offspring; PL: PLxPL offspring; F_1: first-generation hybrids). (a, d, g) Boldness, (b, e, h) doormat and (c, f, i) sociality. Dashed lines indicate 0 on the *x* axis.

Animal Personality in a Context of Adaptive Divergence

Contrary to our predictions, the offspring of the two morphs did not appear to differ strongly in average values (our first aspect of behavioural variation) or in repeatability (our second aspect of behavioural variation) for any of the traits studied. Besides the statistical uncertainty discussed above, these results may also relate to the fact that our experiments were performed presumably during a period when their wild counterparts would not have diverged in habitat use, that is, before the PL young of the year go through their ontogenetic niche shift (see Polverino, Cigliano, Nakayama, and Mehner (2016, 2018) and Herczeg et al. (2013) for examples and counterexamples of conserved personality traits over ontogeny in freshwater fish).

Because of the importance of plasticity in animal personality (Dingemanse, Kazem, Re, & Wright, 2009; Ólafsdóttir & Magellan, 2016), one might expect differences to emerge as a result of diverse environmental conditions encountered during different ontogenetic stages. In Thingvallavatn, the amount of information on the exact timing and duration of the ecological niche shift of the PL juveniles from the benthic to the pelagic habitat, and on the ecology of the juveniles that stay put in the shallow benthic zone, remains too scarce to make strong interpretations (Sandlund et al., 1988). Yet, empirical evidence indicates that some behavioural differences may emerge before this transition phase (Sandlund et al., 1988; Skúlason et al., 1993). For example, direct observations have indicated variation in shoaling behaviour occurring within the surf zone (Sandlund et al., 1988). Moreover, differences in feeding strategies have been observed between PL and SB juveniles reared in common garden conditions (Skúlason et al., 1993). These morphs are also known to develop differences in craniofacial morphology before the onset of exogeneous feeding (Kapralova, 2014; Kapralova et al., 2015). Therefore, variations in behavioural traits related to the ecological differences between the two morphs as adults would have been expected soon after the onset of first feeding.

The only hints of behavioural divergence between the two morphs were observed when considering trait correlations (the third behavioural aspect studied here). Bolder SB charr tended to show more behaviours related to the use of the entrance of the shelter, while these two types of behaviour appeared to be negatively correlated in the PL charr. Given the recent history of the biological system, ca. 10 000 years, these results, although receiving limited statistical support, suggest a scenario of rapid divergence in trait correlations. This trend would contrast with the recent observations of conserved behavioural syndromes, such as among geographically distant populations of field crickets, *Gryllus integer* (Royauté, Hedrick, & Dochtermann, 2020).

Further caution in the interpretation of our results should be taken in light of the importance of nongenetic effects (Herczeg et al., 2013). Several empirical cases suggest that maternal effects can influence the heritability estimates of personality traits (Kasper, Kölliker, Postma, & Taborsky, 2017) or be negligible (Sinn et al., 2006). Unfortunately, our experimental design involving full-

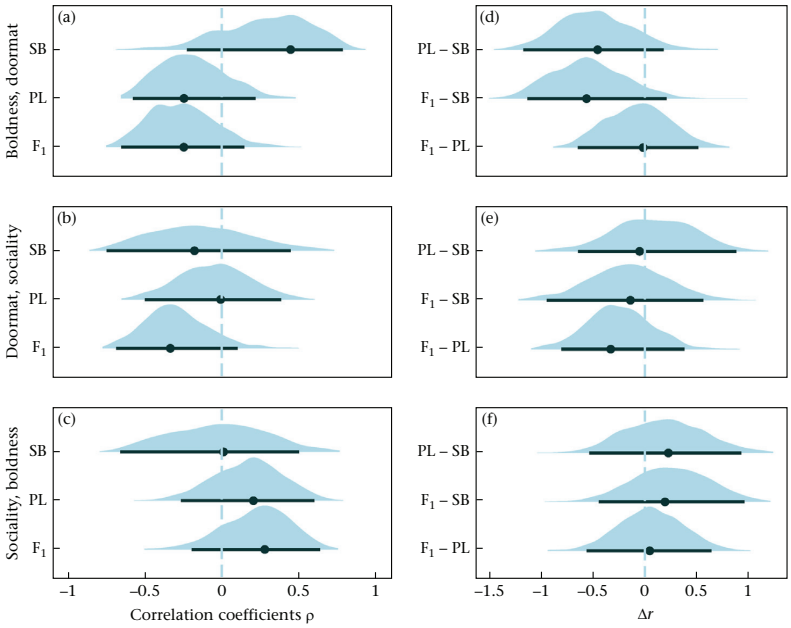


Figure 4. Posterior distributions (blue shading), posterior mode (circle) and 95% credible intervals (horizontal line) of the estimates of (a, b, c) Pearson correlation coefficients between traits at the individual level (behavioural syndrome) for each type of cross, and of (d, e, f) the pairwise differences in the correlation estimates among the types of crosses (0 = no differences between the two groups). SB: SBxSB offspring; PL: PLxPL offspring; F_1: first-generation hybrids. (a, d) Boldness, doormat, (b, e) doormat, sociality and (c, f) sociality, boldness. Boldness and doormat: scores from a factor analysis; sociality: inverse of the average distance to conspecific (cm). Dashed lines indicate 0 on the *x* axis.

sibling families and the merging of reciprocal hybrid crosses into one statistical group did not enable us to obtain thorough characterizations of nonadditive genetic effects, nor to extract components of behavioural variation related to maternal effects. In a meta-analysis, Dochtermann, Schwab, and Sih (2014) reported that a major part of the variation in personality traits was attributed to additive genetic variation in many systems. Thus, studies relying on repeatability estimates may be appropriate for drawing evolutionary inferences in the absence of further information on heritability. Note also that by using the variable related to the family as a proxy of the variance in behaviour related to parental effects (i.e. by replacing V_{cross} by V_{fam} in Eq. (1)), we observed that this component accounted for a very low proportion of the total behavioural variation ($R^2_{(fam)} = 0.01$ [0.00; 0.08]). Although these figures should be interpreted with caution, given our limited number of families, they do indicate that broad-sense nongenetic effects (e.g. variance in egg quality) on behavioural traits might be limited in our study system.

Between-individual differences in behaviour may also originate from permanent environmental effects encountered during ontogeny (Nakagawa & Schielzeth, 2010; Royauté, Garrison, Dalos, Berdal, & Dochtermann, 2019). Such effects are assumed to be mitigated by our common garden set-up. These rearing conditions remain relevant because the offspring of the two morphs are thought to encounter common environmental conditions in the wild before the ontogenetic niche shift of the PL juveniles (Sandlund et al., 1988). However, the limited information available on the ecology of wild juvenile SB and PL charr does not enable us to completely rule out the plausibility of Genetic x Environment differences related to unknown sources of environmental variation.

Hybrid Behaviour and Implications for Speciation

The merging of diverging genomes often results in transgressive or intermediate values of polygenic traits (Albertson & Kocher, 2005). Transgressive or intermediate behaviours in hybrids have recently been proposed as an overlooked source of postzygotic reproductive isolation between diverging populations, including populations with no observed differences in the studied trait (McQuillan, Roth, Huynh, & Rice, 2018; Rice & McQuillan, 2018). While the hybrids from our experiment did not differ from the two pure-morph crosses in their average behavioural responses, they did show some trends towards reductions in the repeatability of two traits (boldness and doormat). Repeatability might therefore be affected by hybrid breakdown (i.e. deficiencies resulting from the negative genetic interactions of the incompatible alleles from diverging genomes, Dobzhansky, 1936) in the same way as for nonbehavioural characters, although the extent to which these apparent changes might be detrimental to the hybrids in the wild remains to be elucidated.

The hybrids also showed a negative correlation between boldness and doormat like the PLxPL offspring but tended to differ from the SBxSB offspring where this correlation was positive. Hybridization is expected to relax trait correlations by breaking down genetic constraints (Seehausen et al., 2014), as observed for morphological characters in hybrids among African cichlids, *Astatotilapia* sp. (Selz, Lucek, Young, & Seehausen, 2014). However, our study did not show signs of relaxed trait correlations in behaviour. Instead, the similarity of the hybrids to one morph suggests at best that these trait correlations might be caused by nonadditive genetic effects. Overall, our study of multiple aspects of juvenile

behavioural variation in diverging morphs of Arctic charr provides limited evidence of rapid behavioural divergence. Furthermore, we did not detect strong signals of potentially detrimental behavioural traits in hybrids.

Acknowledgments

We are very thankful to Alia Desclos for her contribution in conducting the tests and processing the videos and her help in maintaining the rearing set-up. We thank Skúli Skúlason for his comments on the manuscript, and Zophonías O. Jónsson, the members of the 'Arctic charr and salmonid' group of the university of Iceland, and the owner of the farm of Mjóanes, Jóhann Jónsson, for their help during sampling. We are also thankful to Camille Leblanc, Bjarni K. Kristjánsson and Neil Metcalfe for constructive discussions on the experimental design and the conceptual aspects of the study. We thank Kári H. Árnason, Rakel Þorbjörnsdóttir and Christian Beuvard for the organization and maintenance of the rearing facility. This work was entirely funded by the Icelandic Centre of Research, RANNÍS (Icelandic Research Fund grant no.173802–051).

Supplementary Material

Supplementary material associated with this article can be found online at https://doi.org/10.1016/j.anbehav.2021.02.022.

References

Adams, D. C., & Otárola-Castillo, E. (2013). Geomorph: An R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution, 4*(4), 393–399. https://doi.org/10.1111/2041-210X.12035

Albertson, R. C., & Kocher, T. D. (2005). Genetic architecture sets limits on transgressive segregation in hybrid cichlid fishes. *Evolution, 59*(3), 686–690. https://doi.org/10.1111/j.0014-3820.2005.tb01027.x

Arnold, S. J. (1992). Constraints on phenotypic evolution. *The American Naturalist, 140*, 85–107. https://doi.org/10.1086/285398

Barbosa, M., Deacon, A. E., Janeiro, M. J., Ramnarine, I., Morrissey, M. B., & Magurran, A. E. (2018). Individual variation in reproductive behaviour is linked to temporal heterogeneity in predation risk. *Proceedings of the Royal Society B: Biological Sciences, 285*(1870). https://doi.org/10.1098/rspb.2017.1499

Bell, A. M., Hankison, S. J., & Laskowski, K. L. (2009). The repeatability of behaviour: A meta-analysis. *Animal Behaviour, 77*(4), 771–783. https://doi.org/10.1016/j.anbehav.2008.12.022

Benhaïm, D., Ferrari, S., Chatain, B., & Bégout, M. L. (2016). The shy prefer familiar congeners. *Behavioural Processes, 126*(6), 113–120. https://doi.org/10.1016/j.beproc.2016.03.008

Benhaïm, D., Péan, S., Lucas, G., Blanc, N., Chatain, B., & Bégout, M. L. (2012). Early life behavioural differences in wild caught and domesticated sea bass (Dicentrarchus labrax). *Applied Animal Behaviour Science, 141*(1–2), 79–90. https://doi.org/10.1016/j.applanim.2012.07.002

Berry, D. A., & Hochberg, Y. (1999). Bayesian perspectives on multiple comparisons. *Journal of Statistical Planning and Inference, 82*(1–2), 215–227. https://doi.org/10.1016/S0378-3758(99)00044-0

Bollen, K. (2002). Latent variables in psychology and the social sciences. *Annual Review of Psychology, 53*(1), 605–634.

Budaev, S. V. (2010). Using principal components and factor analysis in animal behaviour research: Caveats and guidelines. *Ethology, 116*(5), 472–480. https://doi.org/10.1111/j.1439-0310.2010.01758.x

Church, K. D. W., & Grant, J. W. A. (2018). Does increasing habitat complexity favour particular personality types of juvenile Atlantic salmon, *Salmo salar*? *Animal Behaviour, 135*, 139–146. https://doi.org/10.1016/j.anbehav.2017.11.006

Dahlbom, S. J., Lagman, D., Lundstedt-Enkel, K., Sundström, L. F., & Winberg, S. (2011). Boldness predicts social status in zebrafish (*Danio rerio*). *PloS One, 6*(8), 2–8. https://doi.org/10.1371/journal.pone.0023565

Dall, S. R. X., Houston, A. I., & McNamara, J. M. (2004). The behavioural ecology of personality : Consistent individual differences from an adaptive perspective. *Ecology Letters, 7*(8), 734–739. https://doi.org/10.1111/j.1461-0248.2004.00618.x

Dingemanse, N. J., & Dochtermann, N. A. (2012). Quantifying individual variation in behaviour : Mixed-effect modelling approaches. *Journal of Animal Ecology, 82*(1), 39–54. https://doi.org/10.1111/1365-2656.12013

Dingemanse, N. J., Kazem, A. J. N., Re, D., & Wright, J. (2009). Behavioural reaction norms : Animal personality meets individual plasticity. *Trends in Ecology & Evolution, 25*(2), 81–89. https://doi.org/10.1016/j.tree.2009.07.013

Dingemanse, N. J., Wright, J., Anahita, J. N., Thomas, D. K., Hickling, R., & Dawnay, N. (2007). Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. *Journal of Animal Ecology, 76*, 1128–1138. https://doi.org/10.1111/j.1365-2656.2007.01284.x

Dobzhansky, T. (1936). Studies on hybrid sterility–II. Localization of sterility factors in Drosophila pseudoobscura hybrids. *Genetics, 21*(2), 169–223. https://doi.org/10.1007/BF00374056

Dochtermann, N. A., Schwab, T., & Sih, A. (2014). The contribution of additive genetic variation to personality variation: Heritability of personality. *Proceedings of the Royal Society B: Biological Sciences, 282*(1798). https://doi.org/10.1098/rspb.2014.2201

Ferguson, M., & Noakes, D. L. G. (1982). Genetics of social behaviour in charr (*Salvelinus* species). *Animal Behaviour, 30*, 128–134. https://doi.org/10.1016/S0003-3472(82)80247-9

Garamszegi, L. Z. (2016). A simple statistical guide for the analysis of behaviour when data are constrained due to practical or ethical reasons. *Animal Behaviour, 120*, 223–234. https://doi.org/10.1016/j.anbehav.2015.11.009

García-Berthou, E. (2001). On the misuse of residuals in ecology: Regression of residuals vs. multiple regression. *Journal of Animal Ecology, 70*, 708–711. https://doi.org/10.1046/j.1365-2656.2002.00618.x

Goodchild, C. G., Schmidt, L. M., & Durant, S. E. (2020). Evidence for the 'behavioural character' hypothesis : Does boldness programme disparate antipredator strategies ? *Animal Behaviour, 164*, 123–132. https://doi.org/10.1016/j.anbehav.2020.04.010

Guðbrandsson, J., Franzdóttir, S. R., Kristjánsson, B. K., Ahi, E. P., Maier, V. H., Kapralova, K. H., et al. (2018). Differential gene expression during early development in recently evolved and sympatric Arctic charr morphs. *PeerJ, 6*. https://doi.org/10.7717/peerj.4345

Guðbrandsson, J., Kapralova, K. H., Franzdóttir, S. R., Bergsveinsdóttir, T. M., Hafstad, V., Jónsson, Z. O., et al. (2019). Extensive genetic divergence between recently evolved sympatric Arctic charr morphs. *Ecology and Evolution, 9*(9), 1–20. https://doi.org/10.1101/489104

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. *Journal of Statistical Software, 33*(2), 1–22. https://doi.org/10.1002/ana.22635

Herczeg, G., Ab Ghani, N. I., & Merilä, J. (2013). Evolution of stickleback feeding behaviour: Genetics of population divergence at different ontogenetic stages. *Journal of Evolutionary Biology, 26*(5), 955–962. https://doi.org/10.1111/jeb.12103

Ingley, S. J., & Johnson, J. B. (2014). Animal personality as a driver of reproductive isolation. *Trends in Ecology & Evolution, 29*(7), 369–371. https://doi.org/10.1016/j.tree.2014.04.008

Johnson, J. B., Culumber, Z. W., Easterling, R., & Rosenthal, G. G. (2015). Boldness and predator evasion in naturally hybridizing swordtails (teleostei: *Xiphophorus*). *Current Zoology, 61*(4), 596–603. https://doi.org/10.1093/czoolo/61.4.596

Jónasson, P. M. (1992). The ecosystem of Thingvallavatn: A synthesis. *Oikos, 64*, 405–434. https://doi.org/10.2307/3545062

Kapralova, K. H. (2014). *Study of morphogenesis and miRNA expression associated with craniofacial diversity in Arctic charr (Salvelinus alpinus) morphs* (Ph.D. thesis). University of Iceland. Retrieved from http://skemman.is/item/view/1946/19398.

Kapralova, K. H., Jónsson, Z. O., Palsson, A., Franzdóttir, S. R., le Deuff, S., Kristjánsson, B. K., et al. (2015). Bones in motion: Ontogeny of craniofacial development in sympatric Arctic charr morphs. *Developmental Dynamics, 244*(9), 1168–1178. https://doi.org/10.1002/dvdy.24302

Kapralova, K. H., Morrissey, M. B., Kristjánsson, B. K., Olafsdóttir, G.Á., Snorrason, S. S., & Ferguson, M. M. (2011). Evolution of adaptive diversity and genetic connectivity in Arctic charr (*Salvelinus alpinus*) in Iceland. *Heredity, 106*(3), 472–487. https://doi.org/10.1038/hdy.2010.161

Kasper, C., Kölliker, M., Postma, E., & Taborsky, B. (2017). Consistent cooperation in a cichlid fish is caused by maternal and developmental effects rather than heritable genetic variation. *Proceedings of the Royal Society B: Biological Sciences, 284*(1858). https://doi.org/10.1098/rspb.2017.0369

Kern, E. M. A., Robinson, D., Gass, E., Godwin, J., & Langerhans, R. B. (2016). Correlated evolution of personality, morphology and performance. *Animal Behaviour, 117*, 79–86. https://doi.org/10.1016/j.anbehav.2016.04.007

Leblanc, C. A. L., Benhaïm, D., Hansen, B. R., Kristjánsson, B. K., & Skúlason, S. (2011). The importance of egg size and social effects for behaviour of Arctic charr juveniles. *Ethology, 117*(8), 664–674. https://doi.org/10.1111/j.1439-0310.2011.01920.x

McQuillan, M. A., Roth, T. C., Huynh, A. V., & Rice, A. M. (2018). Hybrid chickadees are deficient in learning and memory. *Evolution, 72*(5), 1155–1164. https://doi.org/10.1111/evo.13470

Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: A practical guide for biologists. *Biological Reviews, 85*(4), 935–956. https://doi.org/10.1111/j.1469-185X.2010.00141.x

Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution, 4*(2), 133–142. https://doi.org/10.1111/j.2041-210x.2012.00261.x

Nosil, P. (2012). *Ecological Speciation*. Oxford, U.K.: Oxford University Press.

Ólafsdóttir, G.Á., & Magellan, K. (2016). Interactions between boldness, foraging performance and behavioural plasticity across social contexts. *Behavioral Ecology and Sociobiology, 70*(11), 1879–1889. https://doi.org/10.1007/s00265-016-2193-0

Olsen, A. M., & Westneat, M. W. (2015). StereoMorph: An R package for the collection of 3D landmarks and curves using a stereo camera set-up. *Methods in Ecology and Evolution, 6*(3), 351–356. https://doi.org/10.1111/2041-210X.12326

Orpwood, J. E., Magurran, A. E., Armstrong, J. D., & Griffiths, S. W. (2008). Minnows and the selfish herd: Effects of predation risk on shoaling behaviour are dependent on habitat complexity. *Animal Behaviour, 76*(1), 143–152. https://doi.org/10.1016/j.anbehav.2008.01.016
Pétursson, H. G., Norðdahl, H., & Ingólfsson. (2015). Late Weichselian history of relative sea level changes in Iceland during a collapse and subsequent retreat of marine based ice sheet. *Cuadernos de Investigación Geográfica, 41*(2), 261–277. https://doi.org/10.18172/cig.2741
Polverino, G., Cigliano, C., Nakayama, S., & Mehner, T. (2016). Emergence and development of personality over the ontogeny of fish in absence of environmental stress factors. *Behavioral Ecology and Sociobiology, 70*(12), 2027–2037. https://doi.org/10.1007/s00265-016-2206-z
Polverino, G., Santostefano, F., Díaz-Gil, C., & Mehner, T. (2018). Ecological conditions drive pace-of-life syndromes by shaping relationships between life history, physiology and behaviour in two populations of Eastern mosquitofish. *Scientific Reports, 8*(1), 1–10. https://doi.org/10.1038/s41598-018-33047-0
Pounder, K. C., Mitchell, J. L., Thomson, J. S., Pottinger, T. G., & Sneddon, L. U. (2018). Physiological and behavioural evaluation of common anaesthesia practices in the rainbow trout. *Applied Animal Behaviour Science, 199*(May 2017), 94–102. https://doi.org/10.1016/j.applanim.2017.10.014
Réale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V., & Montiglio, P. O. (2010). Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions of the Royal Society B: Biological Sciences, 365*(1560), 4051–4063. https://doi.org/10.1098/rstb.2010.0208
Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews, 82*(2), 291–318. https://doi.org/10.1111/j.1469-185X.2007.00010.x
Revelle, W. (2019). *psych: Procedure for Personality and Psychological Research*. Evanston, IL: Northwestern University. https://CRAN.R-project.org/package=psych Version=1.8.12.
Rice, A. M., & McQuillan, M. A. (2018). Maladaptive learning and memory in hybrids as a reproductive isolating barrier. *Proceedings of the Royal Society B: Biological Sciences, 285*(1879). https://doi.org/10.1098/rspb.2018.0542
Roche, D. G., Careau, V., & Binning, S. A. (2016). Demystifying animal 'personality' (or not): Why individual variation matters to experimental biologists. *Journal of Experimental Biology, 219*(24), 3832–3843. https://doi.org/10.1242/jeb.146712
Royauté, R., Garrison, C., Dalos, J., Berdal, M. A., & Dochtermann, N. A. (2019). Current energy state interacts with the developmental environment to influence behavioural plasticity. *Animal Behaviour, 148*, 39–51. https://doi.org/10.1016/j.anbehav.2018.11.013
Royauté, R., Hedrick, A., & Dochtermann, N. A. (2020). Behavioral syndromes shape evolutionary trajectories via conserved genetic architecture. *Proceedings of the Royal Society B: Biological Sciences, 287*. https://doi.org/10.1101/619411
Sandlund, O. T., Gunnarsson, K., Jónasson, P. M., Jonsson, B., Lindem, T., Magnússon, K. P., et al. (1992). The arctic charr *Salvelinus alpinus* in Thingvallavatn. *Oikos, 64*(12), 305–351. https://doi.org/10.2307/3545056
Sandlund, O. T., Malmquist, H. J., Jonsson, B., Skúlason, S., Snorrason, S. S., Jónasson, P. M., et al. (1988). Density, length distribution, and diet of age-0 Arctic charr *Salvelinus alpinus* in the surf zone of Thingvallavatn, Iceland. *Environmental Biology of Fishes, 23*(3), 183–195. https://doi.org/10.1007/BF00004909
Schluter, D. (2001). Ecology and the origin of species. Introductory statement. *Trends in Ecology & Evolution, 16*(7), 372–380. https://doi.org/10.1016/S0169-5347(01)02198-X
Seehausen, O., Butlin, R. K., Keller, I., Wagner, C. E., Boughman, J. W., Hohenlohe, P. A., et al. (2014). Genomics and the origin of species. *Nature Reviews Genetics, 15*(3), 176–192. https://doi.org/10.1038/nrg3644
Selz, O. M., Lucek, K., Young, K. A., & Seehausen, O. (2014). Relaxed trait covariance in interspecific cichlid hybrids predicts morphological diversity in adaptive radiations. *Journal of Evolutionary Biology, 27*, 11–24. https://doi.org/10.1111/jeb.12283
Sih, A., Cote, J., Evans, M., Fogarty, S., & Pruitt, J. (2012). Ecological implications of behavioural syndromes. *Ecology Letters, 15*(3), 278–289. https://doi.org/10.1111/j.1461-0248.2011.01731.x
Sinn, D. L., Apiolaza, L. A., & Moltschaniwskyj, N. A. (2006). Heritability and fitness-related consequences of squid personality traits. *Journal of Evolutionary Biology, 19*(5), 1437–1447. https://doi.org/10.1111/j.1420-9101.2006.01136.x
Sjölander, A., & Vansteelandt, S. (2019). Frequentist versus Bayesian approaches to multiple testing. *European Journal of Epidemiology, 34*(9), 809–821. https://doi.org/10.1007/s10654-019-00517-2
Skúlason, S., Noakes, D. L. G., & Snorrason, S. S. (1989a). Ontogeny of trophic morphology in four sympatric morphs of arctic charr *Salvelinus alpinus* in Thingvallavatn, Iceland. *Biological Journal of the Linnean Society, 38*(3), 281–301. https://doi.org/10.1111/j.1095-8312.1989.tb01579.x
Skúlason, S., Parsons, K. J., Svanbäck, R., Räsänen, K., Ferguson, M. M., Adams, C. E., et al. (2019). A way forward with eco evo devo: An extended theory of resource polymorphism with postglacial fishes as model systems. *Biological Reviews, 94*(5), 1786–1808. https://doi.org/10.1111/brv.12534
Skúlason, S., Snorrason, S. S., Noakes, D. L. G., Ferguson, M. M., & Malmquist, H. J. (1989b). Segregation in spawning and early life history among polymorphic Arctic charr, *Salvelinus alpinus*, in Thingvallavatn, Iceland. *Journal of Fish Biology, 35*(1989), 225–232. https://doi.org/10.1111/j.1095-8649.1989.tb03065.x
Skúlason, S., Snorrason, S. S., Ota, D., & Noakes, D. L. G. (1993). Genetically based differences in foraging behaviour among sympatric morphs of Arctic charr (Pisces: Salmonidae). *Animal Behaviour, 45*, 1179–1192. https://doi.org/10.1006/anbe.1993.1140
Smith, B. R., & Blumstein, D. T. (2008). Fitness consequences of personality: A meta-analysis. *Behavioral Ecology, 19*(2), 448–455. https://doi.org/10.1093/beheco/arm144
Snorrason, S. S., Malmquist, H. J., Jonsson, B., Jónasson, P. M., Sandlund, O. T., & Skúlason, S. (1994). Modification in life history characteristics of planktivorous Arctic charr *Salvelinus alpinus* in Thingvallavatn, Iceland. *Verhandlungen Des Internationalen Verein Limnologie, 25*, 2108–2112. https://doi.org/10.1080/03680770.1992.11900570
Snorrason, S. S., & Skúlason, S. (2004). Adaptive speciation in northern freshwater fishes. In U. Diekmann, H. Metz, M. Doebeli, & D. Tautz (Eds.), *Adaptive Speciation* (pp. 210–228). Cambridge, U.K: Cambridge University Press. https://doi.org/10.1017/CBO9781139342179.012.
The Imagine Source. (2014). IC Capture - image acquisition. Retreived from https://www.theimagingsource.com/support/downloads-for-windows/end-user-software/iccapture/. (Accessed 25 February 2021).
Villemereuil, P. De, Morrissey, M. B., Nakagawa, S., & Schielzeth, H. (2018). Fixed-effect variance and the estimation of repeatabilities and heritabilities : Issues and solutions. *Journal of Evolutionary Biology, 31*, 621–632. https://doi.org/10.1111/jeb.13232
Ward, A. J. W., Thomas, P., Hart, P. J. B., & Krause, J. (2004). Correlates of boldness in three-spined sticklebacks (*Gasterosteus aculeatus*). *Behavioral Ecology and Sociobiology, 55*(6), 561–568. https://doi.org/10.1007/s00265-003-0751-8
Wilson, D. S. (1998). Adaptive individual differences within single populations. *Philosophical Transactions of the Royal Society B: Biological Sciences, 353*(1366), 199–205. https://doi.org/10.1098/rstb.1998.0202
Wolf, M., & Weissing, F. J. (2012). Animal personalities: Consequences for ecology and evolution. *Trends in Ecology & Evolution, 27*(8), 452–461. https://doi.org/10.1016/j.tree.2012.05.001

Appendix

Table A1
Variables measured during the open field test with shelter

Variable name	Description
Angular speed	Absolute angular velocity of the fish (degrees/s), calculated using the following equation: $V_{angn} = \mathrm{RTA}_n(t_n - t_{n-1})^{-1}$ where RTA_n is the relative turn angle of the sampled position n, and t the sampling time. The rate of change in direction was unsigned. The turn angle was calculated as the difference between two subsequent values for the direction of the head. This variable is an index of swimming path complexity. High V_{angn} values can be referred to high levels of vigilance (Benhaïm et al., 2012)
Arena centre: frequency	Number of events involving the fish entering the central zone
Arena centre: time	Total time spent in the central zone (s)
Arena edges: time	Time spent within the marginal zone of the arena (s)
Average distance to shelter	Mean distance between the fish and the shelter (cm)
Latency to leave shelter	Time lag between the removal of the trapdoor and the first exit of the fish (s)
Shelter: frequency	Number of events involving the fish returning to the shelter
Shelter: time	Total time spent inside the shelter (s)
Shelter entrance: frequency	Number of events involving the fish entering the shelter
Shelter entrance: time	Total time spent in the entrance zone in front of the shelter (s)
Total distance	Total distance swam (cm)
Velocity	Mean velocity (body length swam/s)

Table A2
Table of variance of a reduced (1) and full (2) model of variation of weight with size

Model	Formula	Coefficient	Estimate	SE	*t*	*P*
1	Weight ~ 1 + log (length)	Intercept	-1.10	0.06	-17.62	<0.01
		Log (length)	1.18	0.05	23.86	<0.01
2	Weight ~ log (length) + cross + log (length)*cross	Intercept (crossPL)	-1.12	0.15	-7.54	<0.01
		Log (length)	1.19	0.11	10.69	<0.01
		CrossSB	-0.10	0.26	-0.38	0.71
		CrossF_1	0.05	0.17	0.28	0.78
		Log (length)*crossSB	0.08	0.21	0.37	0.71
		Log (length)*crossF_1	-0.03	0.13	-0.21	0.84

The residuals of the reduced model were used as an estimate of body condition. Adjusted $R^2 = 0.86, 0.86$, $df = 91, 87$, $F = 569.5, 111.1$ for Model 1 and 2, respectively.

Table A3
Summary table of the multiresponse mixed-effect model including 'boldness', 'doormat' and 'sociality' as a multiple response (Model 1)

	Effect	Posterior mode	95% CrI			Effective sample size
Fixed effects						
	Boldness	0.14	-0.80	–	1.24	1000
	Doormat	-0.10	-0.94	–	1.05	1000
	Sociality	-0.57	-1.34	–	0.63	1000
	Length	0.04	-0.14	–	0.16	1292
	Boldness*Trial	-0.10	-0.21	–	0.00	1000
	Doormat*Trial	-0.07	-0.18	–	0.05	1000
	Sociality*Trial	-0.10	-0.22	–	0.01	1000
	Boldness*Cross type-SB	-0.60	-2.32	–	0.66	1000
	Doormat*Cross type-SB	-0.49	-1.89	–	1.13	1000
	Sociality*Cross type-SB	0.64	-1.01	–	1.90	868
	Boldness*Cross type-F_1	-0.76	-1.69	–	0.77	1000
	Doormat*Cross type-F_1	0.19	-1.13	–	1.40	1000
	Sociality*Cross type-F_1	0.49	-0.78	–	1.65	1000
	Boldness*Body condition	0.27	-2.75	–	2.49	885
	Doormat*Body condition	-0.93	-3.90	–	1.53	978
	Sociality*Body condition	0.75	-1.66	–	3.39	1000
Random effects						
	Family	0.30	0.129	–	1.09	1000
	Boldness (V_{ind})	0.41	0.266	–	0.66	637
	Doormat*Boldness (V_{ind})	-0.12	-0.267	–	0.06	1020
	Sociality*Boldness (V_{ind})	0.05	-0.086	–	0.21	1208
	Doormat (V_{ind})	0.46	0.282	–	0.72	1248
	Sociality*Doormat (V_{ind})	-0.12	-0.278	–	0.03	1000
	Sociality (V_{ind})	0.37	0.198	–	0.57	885
Residuals						
	Boldness	0.45	0.36	–	0.64	1000
	Doormat*Boldness	0.02	-0.10	–	0.13	1000
	Sociality*Boldness	0.08	-0.05	–	0.17	1000
	Doormat	0.65	0.48	–	0.84	1000
	Sociality*Doormat	-0.10	-0.23	–	0.03	1000
	Sociality	0.60	0.48	–	0.82	1000

V_{ind}: interindividual variance component. Nonoverlapping 95% high posterior density credible interval (95% CrI) were used to detect significant differences between effects. Cross type-PL constitutes the baseline. Sociality is here interpreted as the average distance to conspecifics (higher values relate to less social individuals).

Table A4
Posterior modes and 95% credible intervals (95% CrI) of the fixed effect of the three seperate linear mixed-effect models with each trait as a response.

Response	Effect	Posterior mode	95% CrI		
Boldness					
	Cross type-PL (Intercept)	0.30	-0.37	–	1.41
	Body condition	0.33	-2.55	–	2.43
	Trial	-0.06	-0.43	–	0.21
	Cross type-SB	-0.20	-1.85	–	1.03
	Cross type-F_1	0.06	-1.27	–	1.03
	Trial*Cross type-SB	-0.11	-0.84	–	0.34
	Trial*Cross type-F_1	-0.24	-0.69	–	0.20
Doormat					
	Cross type-PL (Intercept)	0.34	-0.22	–	1.08
	Body condition	-0.88	-3.02	–	1.37
	Trial	-0.21	-0.58	–	0.09
	Cross type-SB	-0.53	-1.67	–	0.47
	Cross type-F_1	-0.23	-1.00	–	0.71
	Trial*Cross type-SB	0.08	-0.36	–	0.78
	Trial*Cross type-Hybrid	0.23	-0.28	–	0.60
Sociality[a]					
	Cross type-PL (Intercept)	4.57	3.67	–	5.30
	Body condition	2.41	-0.56	–	4.39
	Trial	-0.38	-0.77	–	0.00
	Cross type-SB	0.26	-0.96	–	1.80
	Cross type-F_1	0.04	-0.89	–	1.37
	Trial*Cross type-SB	0.10	-0.75	–	0.71
	Trial*Cross type- F_1	0.08	-0.43	–	0.68

[a] Sociality: average distance to conspecifics (higher values relate to less social individuals).

Table A5
Posterior mode, 95% high posterior density credible intervals (95% CrI) and effective sample size of the variance–covariance components and repeatability estimates for the three multiresponse mixed models (one per cross type)

		Posterior mode	95% CrI	Effective sample size
Pure PL				
	Among-individual variance-covariance			
	Boldness	0.58	0.32;1.11	1000
	COV(Boldness, Doormat)	-0.13	-0.44;0.16	1000
	COV(Boldness, Sociality)	0.11	-0.17;0.39	726
	Doormat	0.52	0.30;1.11	1000
	COV(Doormat, Sociality)	0.00	-0.31;0.26	1000
	Sociality	0.52	0.19;0.84	871
	Within-individual variance-covariance			
	Boldness	0.50	0.33;0.77	1000
	COV(Boldness, Doormat)	-0.05	-0.21;0.15	980
	COV(Boldness, Sociality)	-0.10	-0.31;0.08	1000
	Doormat	0.51	0.34;0.81	1000
	COV(Sociality, Doormat)	-0.06	-0.24;0.14	1000
	Sociality	0.67	0.47;1.00	1000
Pure SB				
	Among-individual variance-covariance			
	Boldness	0.66	0.25;1.61	1000
	COV(Boldness, Doormat)	0.16	-0.22;1.02	1000
	COV(Boldness, Sociality)	0.00	-0.60;0.55	1000
	Doormat	0.62	0.21;1.70	1000
	COV(Doormat, Sociality)	-0.08	-0.83;0.42	1104
	Sociality	0.65	0.23;1.62	1000
	Within-individual variance-covariance			
	Boldness	0.34	0.20;0.77	1000
	COV(Boldness, Doormat)	-0.09	-0.35;0.24	1000
	COV(Boldness, Sociality)	0.17	-0.09;0.55	850
	Doormat	0.53	0.30;1.16	1000
	COV(Sociality, Doormat)	-0.19	-0.61;0.16	1000
	Sociality	0.74	0.42;1.42	894

Table A5 (*continued*)

		Posterior mode	95% CrI	Effective sample size
F_1 hybrids				
	Among-individual variance-covariance			
	Boldness	0.50	0.20;0.85	1176
	COV(Boldness, Doormat)	-0.07	-0.42 : 0.11	1195
	COV(Boldness, Sociality)	0.09	-0.11;0.39	1000
	Doormat	0.58	0.21;0.90	1000
	COV(Doormat, Sociality)	-0.14	-0.43;0.11	1000
	Sociality	0.39	0.19;0.80	1000
	Within-individual variance-covariance			
	Boldness	0.57	0.41;0.91	1000
	COV(Boldness, Doormat)	0.10	-0.10;0.31	1000
	COV(Boldness, Sociality)	0.09	-0.09;0.29	1000
	Doormat	0.79	0.52;1.15	1000
	COV(Sociality, Doormat)	-0.07	-0.32;0.11	1000
	Sociality	0.61	0.43;0.97	1000

COV: covariance; PL: planktivorous; SB: small benthic.

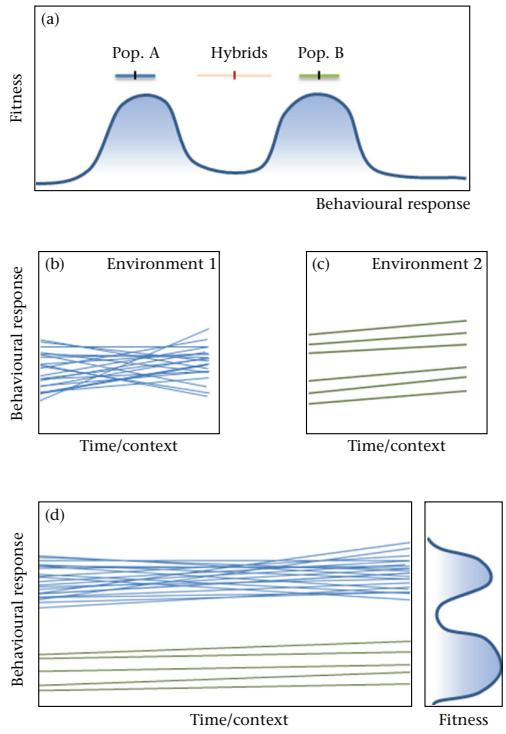


Figure A1. Evolutionary mechanism through which behavioural variation is involved in adaptive divergence. (a) Average behavioural response can diverge between populations as any classic trait under divergent selection, here represented in a rugged-adaptive landscape model (Nosil, 2012). Reproductive isolation can build up as the intermediate or transgressive behavioural responses of hybrids represent a selective disadvantage. (b, c) Different selection regimes can also affect the level of consistent behavioural differences between individuals (i.e. personality) across environments. This can be visualized under a reaction norm approach. While in this example identical average values are favoured in two environments, specialized behavioural types are favoured in environment 2. (d) Complex patterns of adaptive divergence may therefore arise when considering the two aspects of behavioural variations described in (a) and (b).

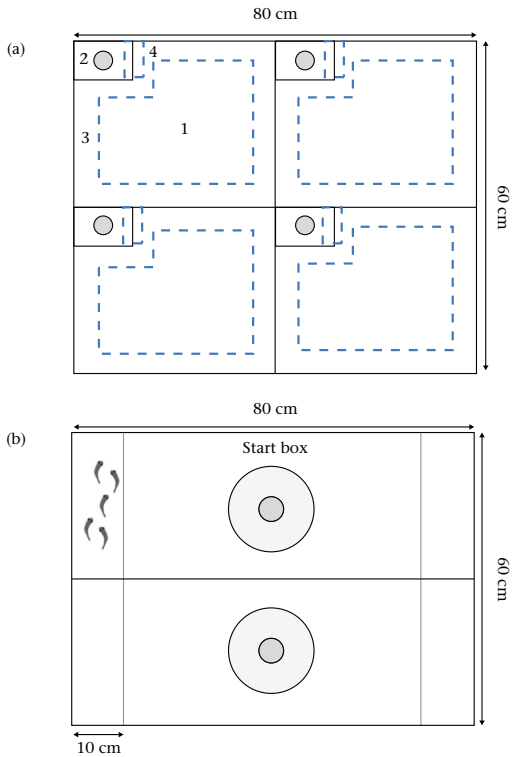


Figure A2. Dimensions of the multiarena set-ups used for the (a) boldness and (b) sociality tests (aerial views). The different virtual zones, delimited by the dashed lines, are: (1) the central zone; (2) the shelter; (3) the marginal zone; (4) the entrance area. Transparent walls are represented by grey continuous lines. A group of congeners is depicted in the upper left compartment in (b). The darker circles are the lids of the shelters and the start boxes, through which the focal fish was introduced.

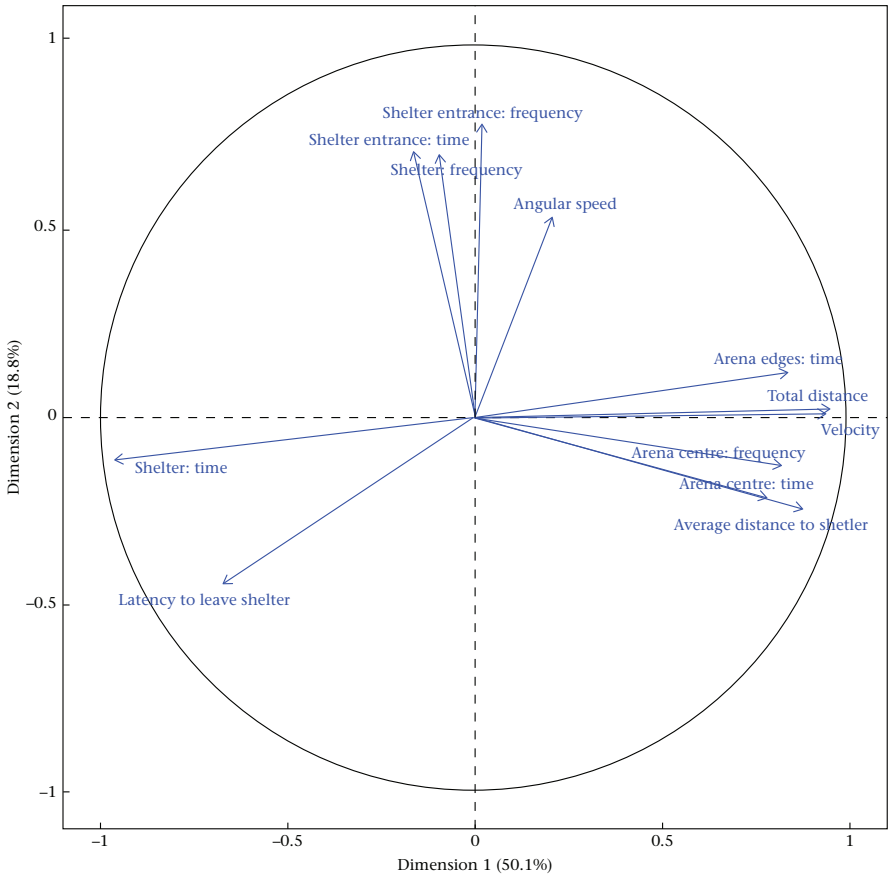


Figure A3. Principal components map of the 12 variables for the boldness test. Dimension 1 is comparable to the boldness latent variable from the factor analysis while dimension 2 is similar to the doormat variable.

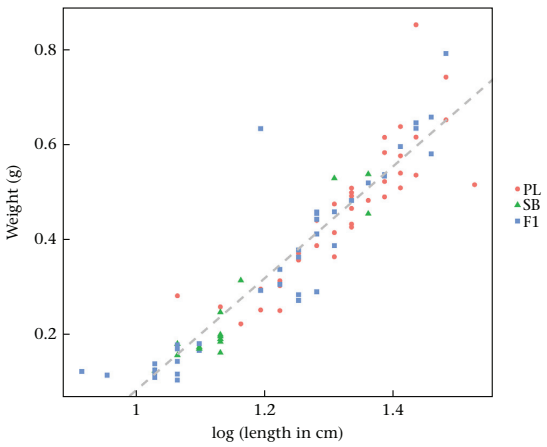


Figure A4. Weight of each specimen regressed over size. The residuals were used in subsequent models as estimates of body condition. PL: planktivorous; SB: small benthic charr; F1: F_1 hybrids. Dashed line: regression line according to Model 1.

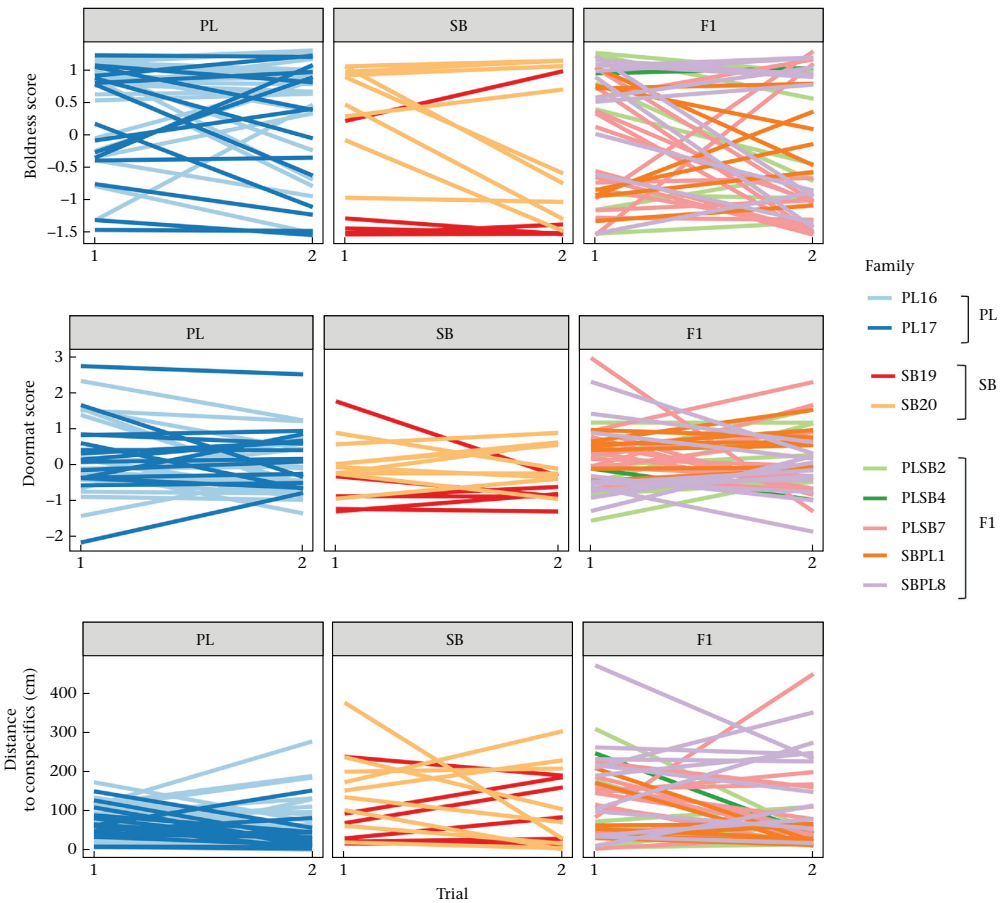


Figure A5. Reaction norms of the behavioural response of each type of cross. Each line links the response of one individual between two replicates of the behavioural test involved. PL: planktivorous; SB: small benthic charr; F1: F_1 hybrids. The average distance to conspecifics constitutes the negative of the sociality trait.

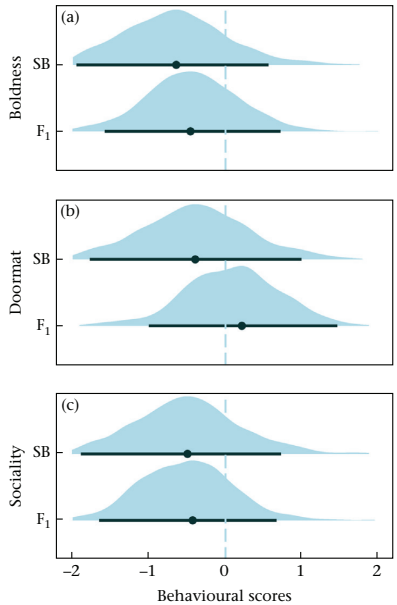
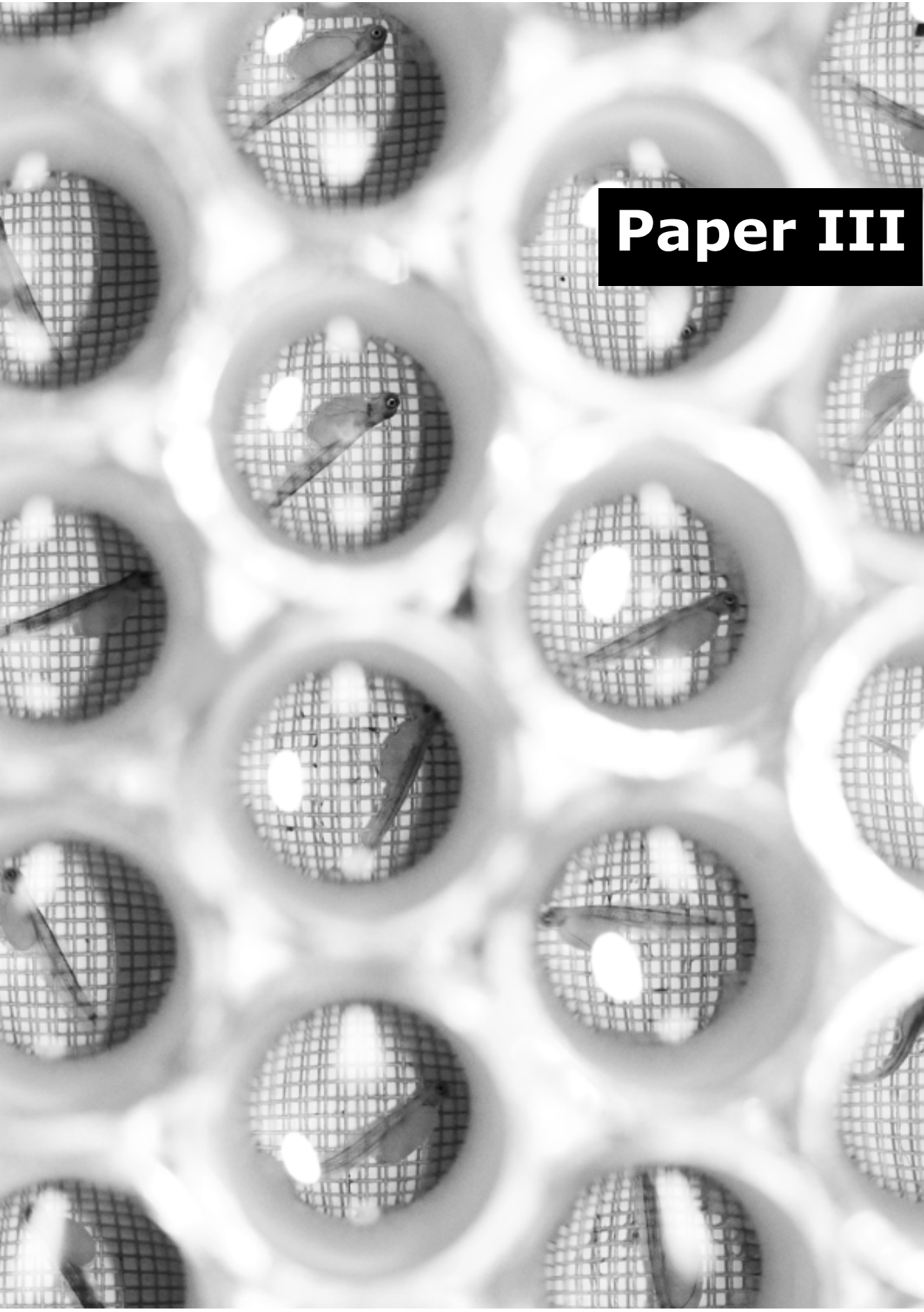


Figure A6. Posterior distributions (blue shading), posterior mode (circle) and 95% credible intervals (horizontal line) of the fixed effect 'type of cross' (SB: SBxSB offspring; F_1: first-generation hybrids) on each trait from the multiresponse model. The PL charr constitutes the baseline (dashed line). The scores are mean-centred and scaled by the unit of standard deviation. Behavioural scores for (a) boldness and (b) doormat: scores of variables from a factor analysis; for (c) sociality: inverse of the average distance to conspecific (cm).



Paper III

Canalization during speciation: lessons from a classic case of resource polymorphism.

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Abstract

The developmental processes buffering phenotypic variability (canalization) are major drivers of evolvability. However, our understanding of the importance of canalization during speciation is rather limited. How hybridization affects phenotypic variability is particularly poorly understood as knowledge on the molecular mechanisms underlying canalisation is sparse underlying canalization. Here, we assessed if/how expression variability evolves in diverging populations. We further investigated how hybridization would affect such divergence in gene expression variability when considering the effects of dominance. Our study system was the Arctic charr morphs (*Salvelinus alpinus*) in Lake Thingvallavatn, a classic case of resource polymorphism involving trophic niches. We estimated gene expression variability in the offspring of two contrasting morphs (benthic/limnetic) and their hybrids reared in common-garden and sampled during two key points of craniofacial development. The two morphs exhibited distinct profiles of gene expression variability for both coding and non-coding RNAs (microRNAs), suggesting that multiple pathways have undergone canalization in either morph. In the hybrids, gene expression variability was substantially affected by maternal effects or was similar to the limnetic morph. Under- and overdominance patterns in expression variability was also observed for a fraction of the genes. Although candidate genes for lower jaw development showed variations in gene expression variability between morphs, these patterns were only partially reflected by morphological variations. In all, we showed that divergence in gene expression variability can evolve rapidly in sympatry. Furthermore, the multiple dominance patterns associated with gene expression variability indicate that many developmental pathways may mediate the effects of hybridization on phenotypic variation.

Significance Statement

Development can modulate trait variations between individuals, thereby influencing how populations evolve and diverge into separate species. Little is known on the developmental mechanisms affecting trait variability (canalization) and on how they affect the population divergence. By analyzing gene expression and morphological variations in embryos of two contrasting Arctic charr morphs, we showed that canalization may rapidly evolve through changes in the noise of gene expression (*i.e.,* variability). Thousands of genes differed in expression variability between the two morphs. However, gene expression variability was under complex dominance patterns involving maternal effects, biases toward one morph and overdominance. Thus, the consequences of hybridization, the emergence of novel traits or hybrid deficiency, may be mitigated or biased by many interacting developmental pathways.

Introduction.

Extensive research efforts have been dedicated over the past few years to understand the evolution of ontogenetic differences between populations, mainly with the aim to unravel the processes of adaptive divergence and speciation (Abzhanov, Protas, Grant, Grant, & Tabin, 2004; Adams & Nistri, 2010; Beck et al., 2019; Bhullar et al., 2012; Cooper et al., 2010; Currey, Bassham, Perry, & Cresko, 2017; Lazić et al., 2014; Parsons et al., 2014; Roberts, Hu, Albertson, & Kocher, 2011; Santos-Santos, Audenaert, Verheyen, & Adriaens, 2021; Skúlason et al., 2019). However, significant knowledge gaps remain on the importance of development in adaptive divergence, notably on the role of canalization, that is, the buffering phenotypic variability in response to genetic and environmental variations (Hallgrímsson et al., 2002; Pesevski & Dworkin, 2020; Waddington, 1942). Alleles or developmental pathways that reduce phenotypic variability spread/evolve in complex ways (Hallgrímsson et al., 2002; Pesevski & Dworkin, 2020; Wagner et al., 1997), and little is known about the processes affecting the evolvability of diverging and hybridizing populations. While hybridization is expected to relax canalization through the breakdown of coadapted alleles or increased heterozygosity, several morphological studies on hybrids or on populations undergoing introgression have revealed contrasting patterns (Ackermann et al., 2006; Alibert, Renaud, Dod, Bonhomme, & Auffray, 1994; Pélabon et al., 2004; Selz et al., 2014). This difficulty to predict the emerging patterns of phenotypic variability through hybridization is congruent with the currently incomplete state of knowledge on the molecular and developmental mechanisms of canalization (Hallgrimsson et al., 2019).

More complexity arises when considering trait dominance and parental effects — hereafter referred to as "dominance" in a broad sense (Albertson, Streelman, & Kocher, 2003; Dagilis, Kirkpatrick, & Bolnick, 2019; Pfennig & Martin, 2009; Thompson, Osmond, & Schluter, 2019). For many traits for example, hybrids of recently diverged populations resemble one of the parents instead of being intermediate, potentially generating "trait mismatches" that complicate predictions about post-zygotic isolation (Thompson, Urquhart-Cronish, Whitney, Rieseberg, & Schluter, 2020). Consequently, it becomes crucial to not only understand how dominance affects developmental processes inducing the divergence of average trait values, but also how it influences phenotypic robustness. Furthermore, it is of special importance to consider the dynamics of phenotypic robustness in the face of gene flow. This can be achieved by investigating whether dominance affects phenotypic robustness in the same way as it affects average trait values, and the consequences for

reproductive isolation or for the maintenance of phenotypic variation between diverging populations.

Gene expression studies are highly suited to address such questions. Using gene expression as a "molecular phenotype" not only enables thorough examination of traits that are hard to quantify (Coolon et al., 2014; Gibson & Weir, 2005; Landry, Hartl, & Ranz, 2007; Pavey et al., 2010) but also provides fundamental information on developmental, genomic and evolutionary mechanisms (Verta & Jones, 2019), especially considering that regulatory changes arise faster than mutations on coding sequences (Satokangas, Martin, Helanterä, & J., 2020) and have substantial phenotypic consequences (Mack & Nachman, 2017). While knowledge is accumulating on how variations in gene expression modulates development at the single-cell levels (Shi, Li, Chen, & Aihara, 2019; Teschendorff & Feinberg, 2021), comparatively very few advances have been achieved for whole organisms. We addressed these knowledge gaps by applying techniques developed in biomedical sciences (Mar, 2019; Simonovsky, Schuster, & Yeger-Lotem, 2019) to infer the evolution of gene expression variability in a context of adaptive divergence. We focused on the expression variability of both coding RNAs and non-coding RNAs. We specifically looked into microRNAs (miRNAs), which are major regulatory elements known for reducing the expression noise of target mRNAs (Siciliano et al. 2013).

We investigated whether and how gene expression variability has diverged between two sympatric Arctic charr morphs (*Salvelinus alpinus*) of lake Thingvallavatn, Iceland. Remarkable cases of polymorphism in Arctic charr have been reported all over the high latitudes of the Northern Hemisphere (Doenz, Krähenbühl, Walker, Seehausen, & Brodersen, 2019; Evgeny, Markev, Grigorii, & Pichugin, 2018; Klemetsen, 2010; Knudsen, Klemetsen, Amundsen, & Hermansen, 2006; Østbye et al., 2020; Pichugin, 2009), and some populations appear to have undergone stronger canalization than others (Parsons, Sheets, et al., 2011). In Thingvallavatn, two of the four described morphs constitute genetically differentiated populations despites wide overlaps in spawning time and location (Guðbrandsson et al., 2019; Kapralova et al., 2011; Kapralova et al., 2013; Skúlason, Snorrason, et al., 1989). The planktivorous charr (PL) is adapted to pelagic life and feeds on zooplankton and emerging chironomids, while the small-benthic charr (SB) forages on benthic invertebrates within the lava matrix. Both morphs have contrasting in head and body shape (Fig. 1a, b). They also differ in life-history and parasites (Jonsson et al., 1988; Sandlund et al., 1992; Snorrason et al., 1994), and have evolved genetically based differences in growth (Jonsson et al., 1988), craniofacial development (Kapralova et al., 2015; Parsons et al., 2010) and foraging strategies (Skúlason et al., 1993). Both morphs occupy highly specialized niches (Malmquist et al., 1992), but the SB-charr are seemingly more morphologically derived than PL-charr compared to the anadromous ancestor (Parsons et al., 2010). The SB- and the PL-charr exhibit complex dissimilarities in morphological plasticity and in variance reduction of head and body shape over ontogeny, which, altogether, suggest intricate differences in canalization (Parsons et al., 2010). The two morphs are reproductively isolated (Guðbrandsson et al., 2019) and F_1 hybrids are rare (Brachmann et al., 2021). Although viable hybrids can be reared in laboratory conditions, recent findings point towards dominance in morphology, growth and personality syndromes, indicating that postzygotic isolation might have evolved between the two morphs through trait mismatches (De la Cámara et al., 2021; Horta-Lacueva, Benhaïm, Morrissey, Snorrason, & Kapralova, 2020; Horta-Lacueva, Snorrason, Morrissey, Leblanc, & Kapralova, 2021).

We estimated mRNA and micro-RNA (miRNA) expression variability in embryos of SB- and PL-morphs and their reciprocal hybrids reared in common garden conditions, focusing on a timeframe when cartilage components of the feeding apparatus are developing (Kapralova et al., 2015). Firstly, we tested for differences in gene expression variability between the two morphs. Dominance underlying these differences in phenotypic robustness was estimated by quantifying intermediate, increased or reduced gene variability in F_1 hybrids (Figure 1e-h). Secondly, we tested whether similar patterns of dominance as observed for gene variability occurred at the level of average gene expression. Like phenotypic robustness, dominance in average gene expression should be revealed through the proportion of intermediate, over- or under-expressed genes in F_1 hybrids.

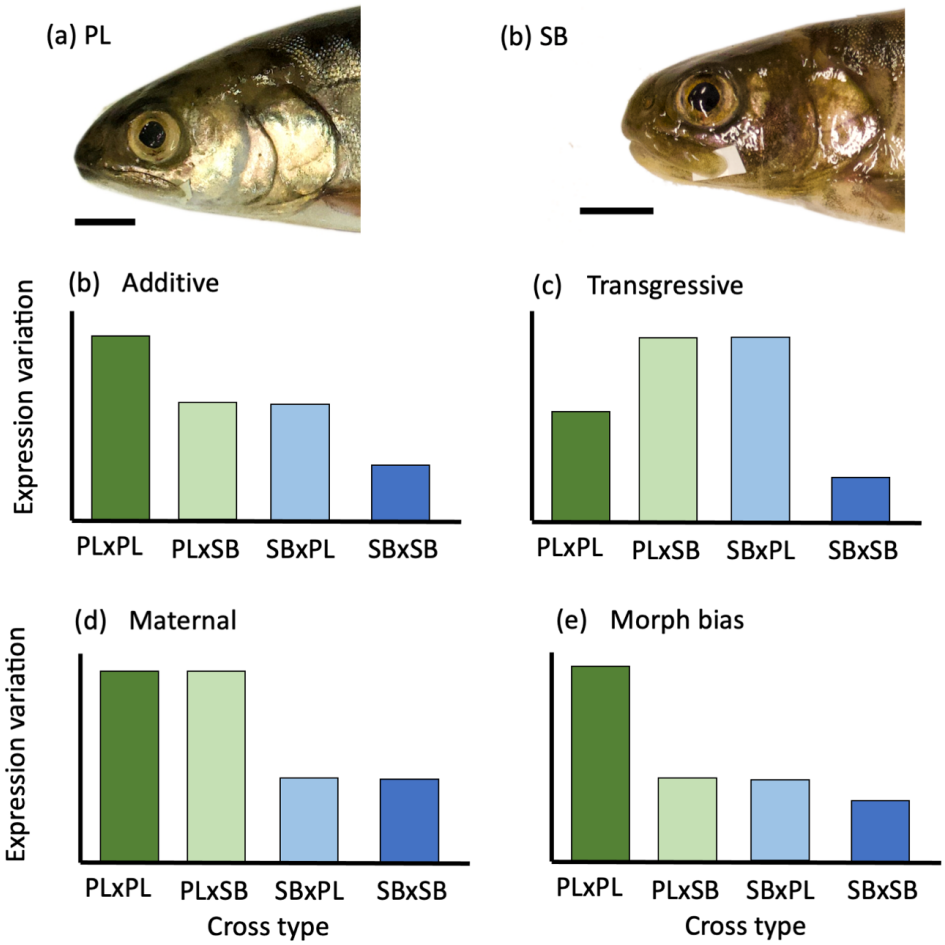

Fig. 1. Expected patterns of gene expression variability. (a-b) External head morphology of mature (a) PL-charr and (b) SB-charr. Background cropped; horizontal bar: 1cm. (c-f) Classification of expression patterns in a scenario with stronger canalization in the SB-morph. Case of transgressive expression involving reduced expression variability in hybrids not shown. Cross type: maternal morph x paternal morph. PL: Planktivorous, SB: Small benthic.

Results.

1. Sympatric morphs show extensive differences in expression variability.

We characterized the gene expression variability profile of each cross type using Local Coefficients of Variation (LCVs; 34). Importantly, we observed similar profiles of expression variability in mRNA and in miRNA (Fig. 2,3). For both coding and non-coding RNAs, genes with covarying expression variability clustered and constituted hierarchical differences among sample groups based on developmental time points and cross type. First, the samples clustered according to the maternal morph (*i.e.,* the PLxSB hybrids clustered with the PLxPL offspring, and the SBxPL hybrids clustered with the SBxSB offspring). Then, the pairs of developmental time points clustered within cross types. However, we did not observe variations in overall gene variability amongst cross types (Fig. S1-S4).

In the mRNA dataset (Fig. 2), we extracted 10 clusters of genes covarying in expression variability: clusters 1, 5 and 7 contained 4390 genes with maternal patterns of expression variability, clusters 2 and 4 had 2719 genes with PL-biased expression in hybrids, and 2445 genes from Clusters 3 and 6 showed transgressive variability in at least one hybrid cross type. For all cross types, 2096 genes had low expression variability (Cluster 8), and 4271 genes had high variability (Clusters 9 and 10). Functional analyses indicated that the genes with maternal patterns of expression variability were enriched in GO terms associated with gene regulation (Clusters 1, 5, 7) but also with head and brain development (Cluster 7). GO terms of genes showing transgressive expression variability in the SBxPL hybrids were associated with translation, immunity and metabolism (Fig. 2b, Table S1). Finally, genes with higher expression variability in SBxSB than in all the other cross types were associated with muscle development, notably in the pharyngeal skeleton (Cluster 2) and with gene expression regulation (Cluster 4).

We also extracted 10 clusters from the miRNA dataset (Fig. 2,3): clusters 4 and 5 revealed maternally controlled expression patterns in 270 genes, clusters 7, 8 and 9 showed 144 genes with a complex pattern with differences in expression variability between the two pure morph crosses in the PLxSB hybrids being similar to the PL-morph while the SBxPL hybrids had intermediate variability. In all cross types, clusters 1 and 2 contained 293 genes with low expression variability, clusters 6 and 10 had 132 highly variable genes, and cluster 3 had 164 with intermediate variability. miRNAs from all the clusters belonged to families expressed in various tissues, brain included (Table S4). No strong inferences could be made about the biological processes affected by miRNA variability: various GO terms, each associated with only a few target genes of miRNAs, were observed in all clusters (Table S2). However, we observed trends for enrichment in GO terms associated with eye and nervous system development in cluster 1 (low expression variability in all cross types), with heart development in cluster 2 (low expression variability in all cross types), with eye and vascular development in cluster 4 (maternal pattern of expression), with the adrenomedullin pathway in clusters 4 and 5 (maternal pattern of expression), and with nervous system development and epithelial cell proliferation in clusters 8 (high variability, though significantly lower variability in SBxSB) and 10 (high variability, although trend for lower variability in SBxSB).

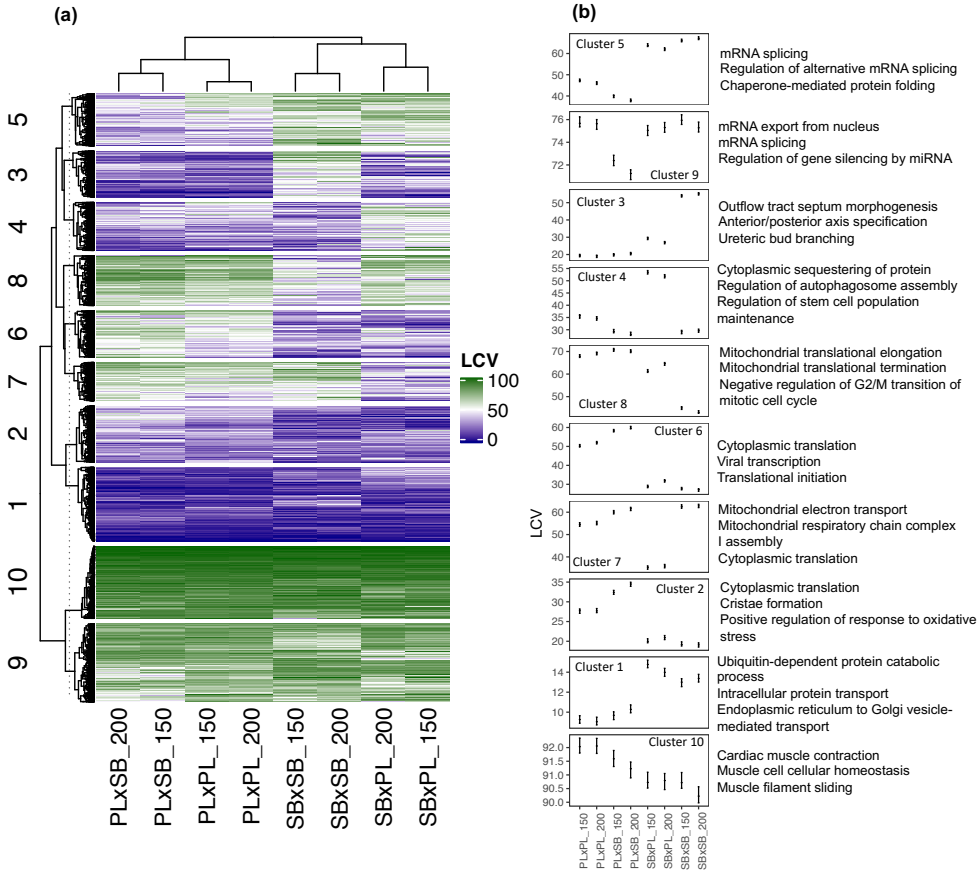

Fig. 2. Variation in mRNAs LCV scores. (a) Heatmap of LCV scores, ranging from 0 (no gene expression variation) to 100 (high gene expression variation). (b) LCV estimates (posterior modes and 95% CrIs) of the expression variability clusters and associated top-3 GO names.

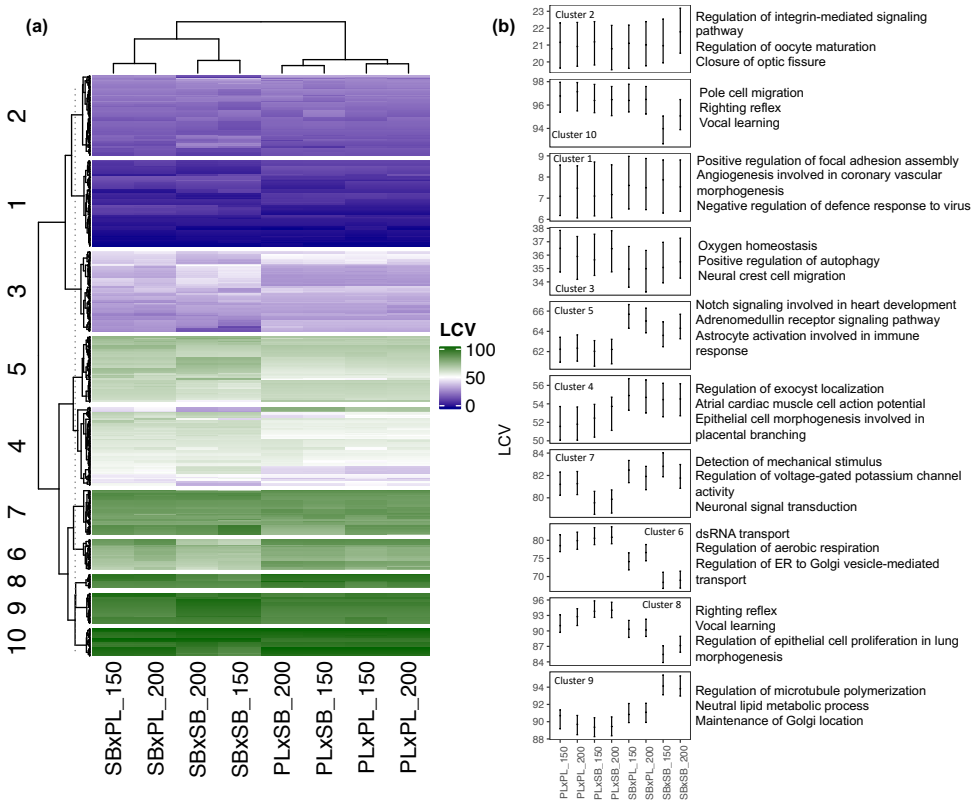

Fig. 3. Variation in miRNAs LCV scores. (a) Heatmap and (b) LCV estimates (posterior modes and 95% CrIs) of the expression variability clusters and associated top-3 GO names. LCV scores range from 0 (no gene expression variation) to 100 (high gene expression variation).

2. Dominance also prevails for average gene expression.

Besides gene expression variability, we also investigated differences in average gene expression between cross types. We observed differential expression between pure morph crosses at both time points ($150\tau_s$, and $200\tau_s$). Only 25 genes were differentially expressed at $150\tau_s$ between the SBxSB and PLxPL crosses, whereas 7824 were differentially expressed at $200\tau_s$ (adjusted P <0.1). Thus, we only used the $200\tau_s$ time point to analyse and discuss general trends (*i.e.,* functional analyses and overall dominance).

At $200\tau_s$, the majority of differentially expressed genes were unique to the pure morph contrast, suggesting intermediate expression in the hybrids (Fig. 4). Much fewer genes were differentially expressed between each hybrid cross types and the cross type of their maternal morph than with the cross type of the alternate maternal morph, which pointing towards substantial maternal effects. The differentially expressed genes between PL-offspring and either hybrid cross types were enriched for GO terms related to metabolism, immunity and mitochondrial DNA inheritance (Table S3). The differentially expressed genes between SBxSB crosses and either hybrid type were enriched for GO terms associated with muscle development (Table S3).

To further explore the extent of maternal effects in overall gene expression, we tested whether the proportion of differentially expressed mRNA genes were lower in the contrasts between pure morph cross types and hybrids with the same maternal morph than between pure morph cross types and the hybrids with a different maternal morph. Such pattern was only detected at $200\tau_s$ in the contrasts involving the reciprocal hybrids and the SBxSB crosses (Table 1).

At $150\tau_s$, one gene was over-dominant (splicing factor U2AF subunit), and one was under-dominant (cytochrome c oxidase subunit) but neither under- nor overdominance expression was detected at $200\tau_s$. While 68 genes showed a maternal pattern of expression at $200\tau_s$, none were detected at $150\tau_s$. We identified biased expression in hybrids towards the PL-morph in 12 genes at $150\tau_s$ and in 38 genes at $200\tau_s$. The genes with maternal and PL-biased expression in hybrids were enriched in GO terms associated with morphogenesis in general and muscle development in particular (Table S5). Overall, the differentially expressed genes, including the ones showing maternal and PL-biased patterns of inheritance were scattered throughout the genome at not found in specific regions of differentiation (Table S6, Fig. S5).

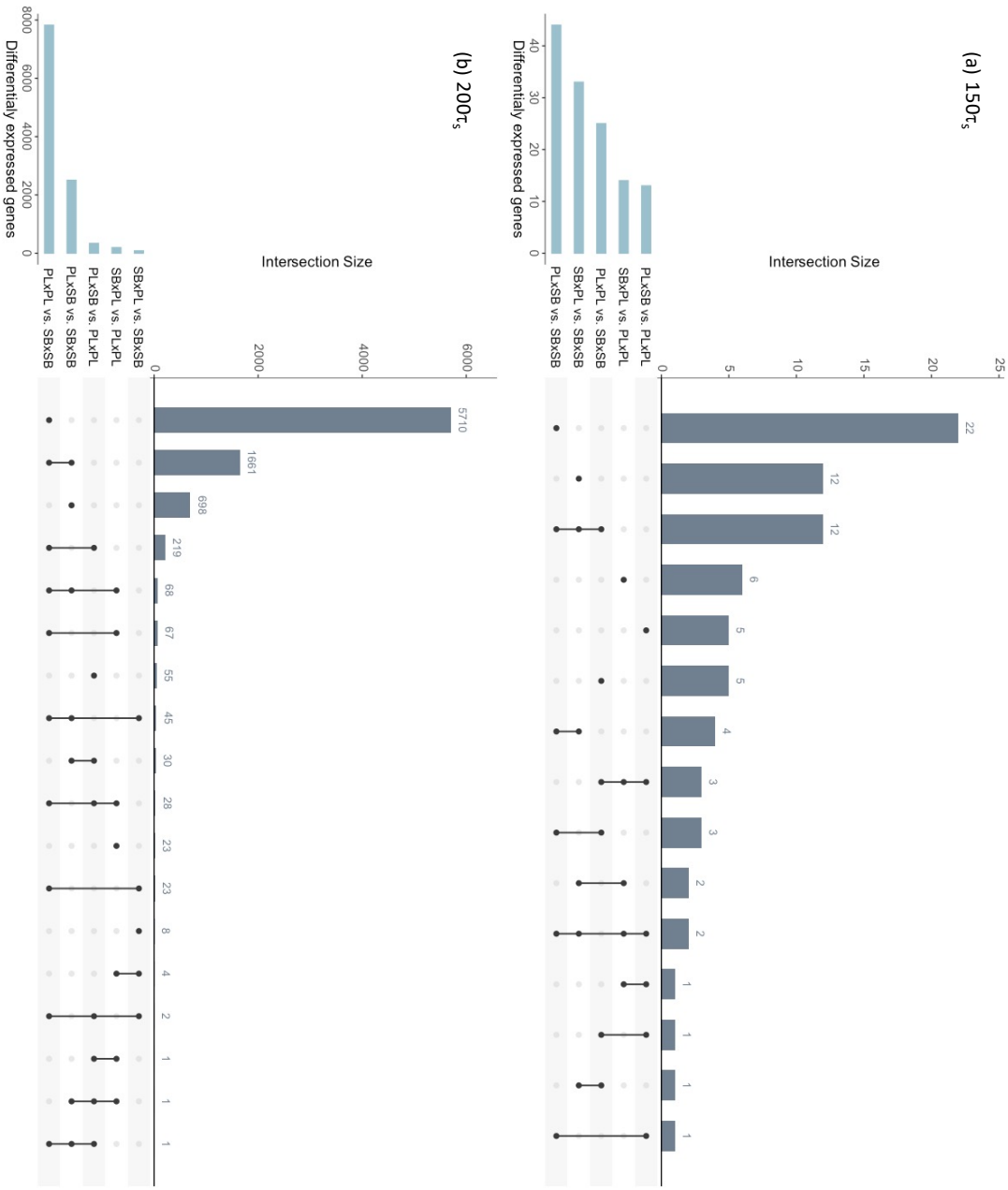

Fig. 4. Intersections between the sets of differentially expressed mRNAs in each cross type comparison, at (a) $150t_s$ and (b) $200t_s$.

Similar results on average gene expression were observed when analysing the miRNA data. Like in mRNAs, differential expression between pure morph crosses was detected at both developmental time points but most of the variation appeared at $200t_s$. MiRNAs from 6 families differed in expression between the two pure morph crosses at $150t_s$ (Table 2). Of those, four families (miR-100, miR-181, miR-34, miR-816) contained differentially expressed genes in more than two cross types (Fig. 3). Two of these families included miRNAs with putative roles in brain development (miR-100 and miR-181; 59, 60) showing PL-biased expression in hybrids. Genes of the miR-34 family — a family involved in brain development (Soni et al., 2013) — were overexpressed in SBxSB compared to both hybrid cross types, and exhibited nonsignificant but substantially very large fold changes between the SBxSB and the PLxPL cross types. Genes of the miR-8160 family were also overexpressed in hybrids, although differences between PLxSB hybrids and the PLxPL offspring were not significant. The role of miR-8160 during development is not currently known.

At $200t_s$, miRNAs from 32 families were differentially expressed between the two morphs, one of those (miR-1) showing a maternal pattern of expression. Members of these families are expressed in neuronal structures, in epidermal tissues and the pharyngeal arches during zebrafish development (Table 3). Note that the predominance of these organs in our dataset may reflect literature biases. For example, there was no observed difference in the proportions of miRNAs reported to be expressed in brains tissues between our set of differentially expressed miRNAs and the full reference dataset of Wienholds and colleagues (2005) (X^2= 1.82; df = 1; P = 0.18).

The target genes of the differentially expressed miRNAs between pure morph crosses were enriched in GO terms associated with numerous biological processes, including eye development and immunity at $150t_s$, and eye and enteric development at $200t_s$ (Table S7).

3. Candidate genes.

We studied gene expression variability in candidate genes involved in the development of the jaw lever systems for mouth opening, a key character determining alternative adaptive feeding modes (Wainwright & Richard, 1995). Briefly, mechanical properties of the lower jaw are realized by a lever system in which the relative shapes of the articular bone and the dentary modulate a trade-off between biting force and velocity, thereby determining suction vs. biting performance (Fig. 6a). Manipulating or crushing feeders are expected to exhibit more effective arm lever for jaw opening compared to suction feeders like planktivorous fishes, for which velocity primes over biting force. We focused on Bone Morphometric Protein 4 (*Bmp4*) and Patched1 *(ptch1),* two candidate genes involved in trophic specialisation and adaptive divergence via the extension of the articular bone, as shown from case studies on mbuna cichlids (e.g., *Labeotropheus fuelleborni.* and *Maylandia zebra*) and from validation experiments in zebrafish, *Danio rerio* (R. Craig Albertson, Streelman, Kocher, & Yelick, 2005; Roberts et al., 2011). *Ptch1* is also directly involved in canalization as regulatory changes among *L. fuelleborni* (biting feeder) and *Tropheops* (generalist) cichlids affect the strength of the plastic response of jaw development to benthic/limnetic foraging environments (Parsons et al., 2016). The role of Bmp4 expression also appear to be conserved among vertebrates, as shown by its major importance in the development of specialised trophic cranial shapes in birds (Abzhanov et al., 2004; Parsons & Albertson, 2009), so Bmp4 expression is likely to have relevant effects on cranial development in Arctic

charr. Focusing on the $200t_s$ time point, we first tested for differences in expression variability among cross types. We observed higher expression variability for *ptch1* in the SBxSB than in the PLxPL crosses (Table 3). In the hybrids, *ptch1* expression variability appeared to be driven by the paternal morph. *Bmp4* expression variation was too low to estimate LCVs in any cross type, suggesting highly conserved expression in this gene.

We then tested for the presence of miRNA target sites in the 3' untranslated regions (3'UTR) of the candidate genes, which could represent another mechanism buffering the variably of their protein products. We identified putative matches between *Bmp4* 3'UTR and three miRNAs: miR-101, miR-21a-2-3, and miR-459-5p (Table S8). miR-101 was absent from our miRNA expression dataset while miR-21a-2-3, and miR-459-5p did not show expression variability and were not differentially expressed between cross types (adjusted $P > 0.5$, $\log_2$ fold change <0.1). We did not identify putative miRNA target sites in *ptch1*.

Finally, we tested for average expression differences among cross types in *bmp4* and *ptch1*. We expected higher expression in both genes in the SB-charr (biting/manipulating feeder) compared to the PL-charr (suction feeder). Surprisingly, we observed higher expression in both genes in the PLxPL offspring compared to the SBxSB offspring while gene expression in hybrids appeared to be intermediate or biased towards the PL-morph (Fig. 5, detailed numeric results in Table S9). Altogether, these results suggest that despite consistent differential expression among cross type for *bmp4* and *ptch1*, divergence in gene expression variability might have evolved in *ptch1* only but is unlikely to be related to regulation by miRNAs.

4. Phenotypic variation.

We used linear measurements of the jaw lever system to verify the consistency of the observed expression variations in *bmp4* and *ptch1* with the morphological phenotype. Using stained free-swimming charr embryos at ca. 460τs (*i.e.*, during ossification of the lower jaw but before the influence of feeding activities), we did not report differences among cross types in the length ratio of the jaw opening in-lever arm length over the out-levers arm (I/O, Fig. 6b). However, the α angle between *I,O* at the attachment of the interoperculo-mandibular ligament was higher in the SBxSB offspring than in the PLxPL offspring, and was intermediate in the PLxSB hybrids (Fig 6d).

Finally, we analyzed the residual variance of I/O and *α* estimates as proxies of phenotypic variability. We did not observe differences between pure morph crosses, but the residual variance of *I/O* was lower in the PLxSB than in the PLxPL and the SBxSB offspring (Fig6c, e), which points towards reduced phenotypic variability in the hybrids.

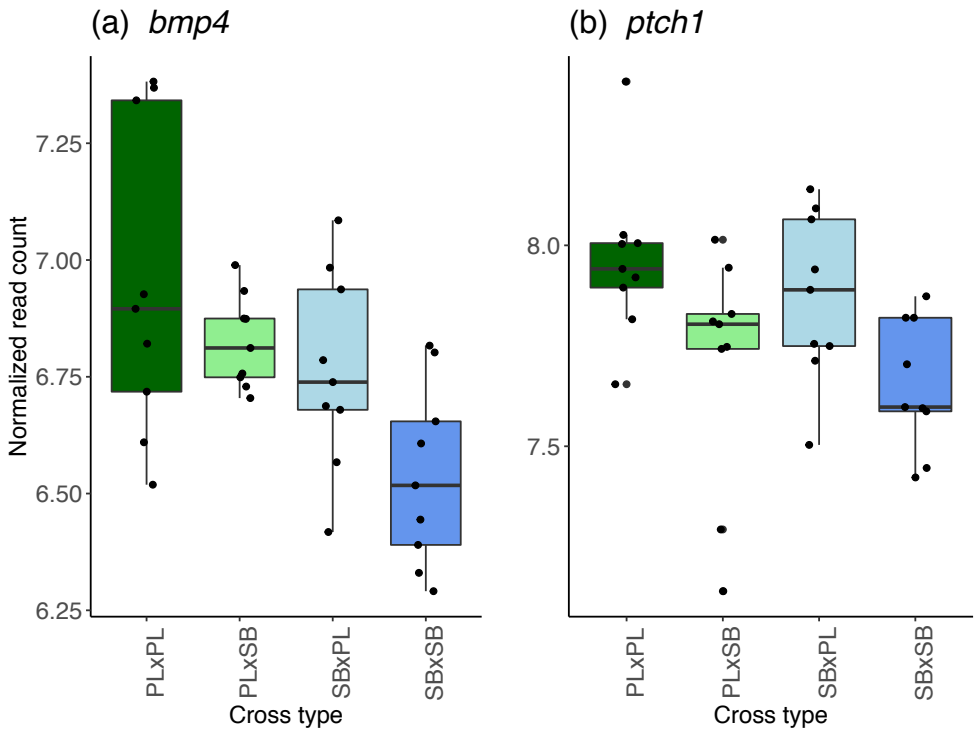

Fig. 5. Normalized read counts of (a) *bmp4* and (b) *ptch1* in each cross type. Horizontal line: median; lower and upper hinges: first and third quartiles; upper and low whiskers: largest and lowest values before 1.5*inter-quartile range. Note the difference between the y-axes.

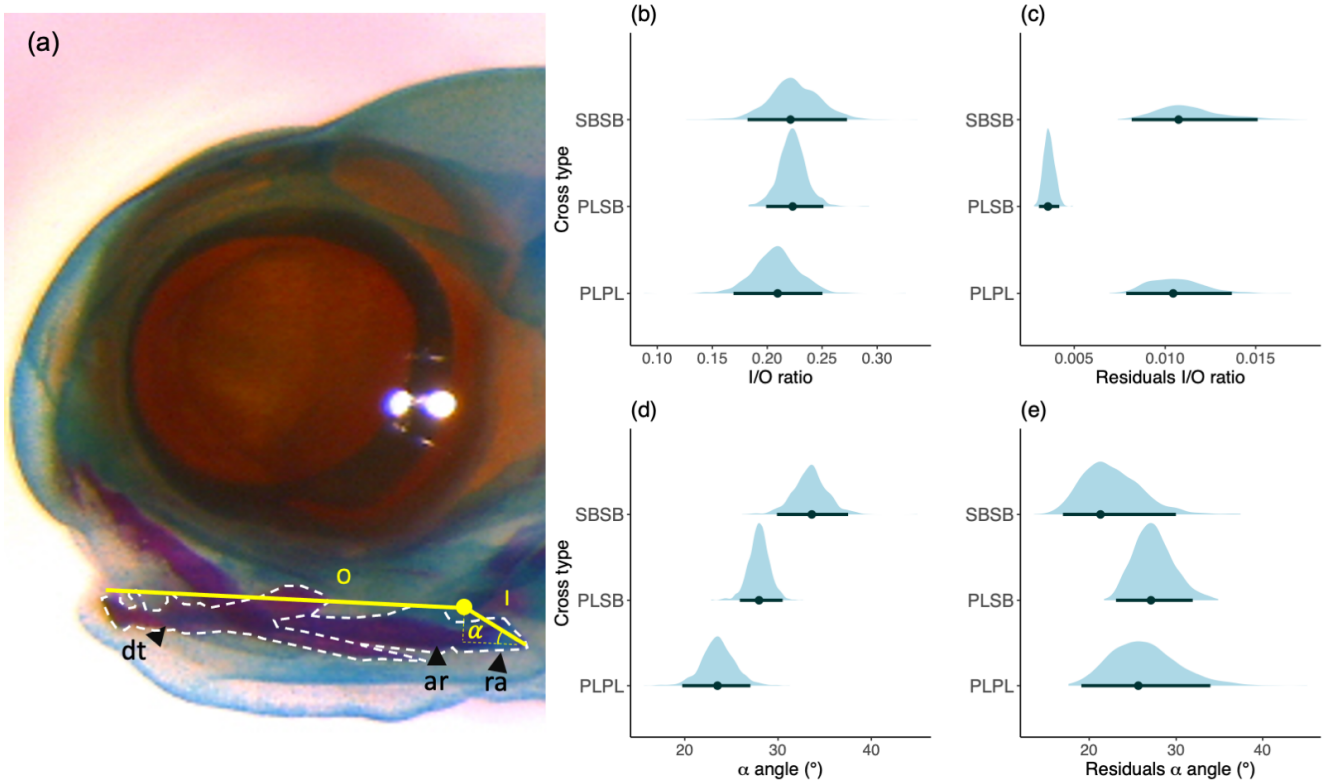

Fig. 6. Variation in the oral jaw lever system among PLxPL, SBxSB and PLxSB offspring. (a) Stained free-swimming charr embryo (ca. 460τ_s) with almost completely ossified dentary (dt), articular (ar) and retroarticular process (ra). (d-e) Posterior modes (dot), posterior densities (blue shade) and 95% CrIs (error bars) of the estimates of the in-lever arm length/out-lever arm length ratio (b) and α (d), and of their respective residual variances (c,e). O: out-lever arm, I: in-lever arm for jaw opening. Dashed line: bones outline.

Discussion.

1. Rapid divergence in gene expression variability.

The compelling patterns of gene expression variability we observed in common-garden reared embryos depict a complex picture involving changes in many traits or developmental pathways, potentially leading to the multifarious differentiation of canalized phenotypes during adaptive divergence. This view is mainly supported by the multiple clusters of genes covarying in expression variability and differing among cross types, remarkably exceeding the expression variation attributed to developmental timing. Most of these clusters showed maternal biases in expression, many others were biased toward the PL-morph and some exhibited over- and underdominance in hybrids. This trend for non-additive expression variability inheritance was substantiated by the consistency of expression profiles from two datasets involving coding and noncoding RNAs. More complexity in gene expression variability and canalization was revealed with the expression study of the candidate genes *bmp4* and *ptch1*. While we reported overexpression for both genes in PL-progeny compared to SB-progeny, gene expression variability was very low in *bmp4* expression in all the cross types, and *ptch1* expression was more variable in SB- than in PL-embryos. The expression of the two genes to some extent reflected the variations observed in the mechanical properties of the lower jaw, the latter indicating differentiation in average trait values but not in canalization between the two morphs. In other words, these results highlight a loose relationship between the observed gene expression variability and phenotypic robustness.

The different clusters of expression variability and their associated GO processes revealed that various pathways or traits may have undergone canalization in either morph. The variations among cross types in gene expression variability were associated with nonadditive inheritance, mostly owing to maternal effects and biases towards the PL-morph. Nonadditive effects on gene expression have been reported in many populations undergoing adaptive divergence or domestication (for salmonids, see 73–75). However, the occurrence and the importance of nonadditive effects is highly variable, even among closely related species, likely because of genetic architecture specificities (Bougas et al., 2010). Furthermore, most of these studies deal with *average* expression, which provides limited information about expression *variability.*

2. Implications of gene variability for evolvability.

The high evolutionary potential of gene expression variability from our data is contrasting with the current view on the developmental origins of phenotypic variation. Nonlinearity in genotype-phenotype maps has been proposed as a parsimonious explanation of the evolution of phenotypic variability (Hallgrímsson et al., 2002), which was recently supported by experimental studies on single genes. For example, enhanced phenotypic variation can result from decelerating gene expression dose-responses curves, thereby producing the most distinct phenotypes for the same gene expression difference at the lowest gene expression levels, as observed in mouse with the effects of *Fgf8* on midfacial shape (Green et al., 2017), or with *Wnt9b* on mouth clefting (Green et al., 2019). Yet, the transcriptome scale snapshots provided by our study suggest important additional mechanisms modulating canalization. In our study, those mechanisms take the form of direct changes in gene expression variability and appear to affect a multitude of genes.

The developmental implications of such changes in gene expression variability may be manyfold, but important insights can be gained through conceptual models. Waddington's epigenetic landscape, which depicts the funneling of developmental processes into valleys whose steepness represents resistance to developmental variation (Hallgrímsson et al., 2002; Waddington, 1942), is an especially powerful metaphor to envision the role of gene expression in canalization. In a context of adaptive divergence, the stochastic developmental processes acting within individuals can drift towards distinct coordinates of the developmental space, which ultimately correspond to phenotypes approximating contrasting fitness optima. Gene expression variability can be conceptualized as the potential energy affecting the trajectory of these developmental processes across the epigenetic landscape (*i.e.*, the steepness of valleys). If hybridization increases gene expression variability (as observed in hybrids between *Coregonus clupeaformis* incipient species, for example 73), such metaphoric landscapes would "flatten", resulting in wide developmental opportunities with potentially diverse evolutionary consequences (e.g., maladapted phenotypes, increased phenotypic novelty, or high resilience to incompatibilities). However, we showed that increased expression variability is not a systematic outcome of hybridization, at least regarding first generation hybrids. Rather, maternal effects and morph biases predominate, suggesting a state of canalization in hybrids that can be conceptualized as a composite picture of multi-layered landscapes, most of those tending towards the values observed in one morph. On the one hand, the phenotypic outcomes of such multivariate landscapes may pertain with the hybrid trait mismatch commonly used to refer to average phenotypic values. On the other hand, phenotypic robustness may be modulated by the interconnectivity of developmental pathways (e.g., from gene networks, developmental constraints, or tissue interactions). In this model, phenotypic effects resulting from the disruption of the "room of maneuver" of a developmental pathway (due to mutations or genetic breakdowns through hybridization) could be buffered – or accentuated – by the state of canalization of other co-acting pathways.

4. Evolutionary implications.

Our study provides compelling evidence for the rapid evolution of gene expression variability among diverging populations. However, the role of genetically based differences in gene expression variability for speciation remains to be clarified, especially when considering scenarios of sympatric divergence. Hybridization can produce unviable phenotypes contributing to reproductive isolation (Coyne & Orr, 2004), or may increase phenotypic variation, ultimately facilitating adaptive diversification (Selz, Thommen, Pierotti, Anaya-Rojas, & Seehausen, 2016; Stelkens et al., 2009). Our results suggest that increased (gene expression) variability in hybrids may not always be the rule. To the contrary, the predominance of maternal effects, morph-biases and, to a lesser extent, transgressive variability are indicative of a unique combination of trait variances in the hybrid phenotype. Whether this assemblage of trait variabilities is detrimental (e.g., by producing trait mismatches 81) or facilitates diversification may depend on ecological opportunities. In the case of Thingvallavatn, fitness estimates related to the expression of genes identified in our study are not available. However, *average* gene expression can give further indications about the condition of F_1 hybrids.

Considering the average gene expression, the predominance of maternal biases at the scale of the transcriptome in the one hand, and the intermediate or PL-biased expression of candidate genes involved in trophic adaptations (e.g., *ptch1* and *bmp4*) in the other hand,

support the view of trait mismatches. This is further supported by the identification of PL- and maternally biased miRNAs expression with putative roles in the nervous system (miR-100, miR-181) and muscle (miR-1) development. Therefore, hybrids exhibiting phenotypic values that are closer to one morph (and eventually present some transgressive characters) might not perform as well as the parental morphs in either of their respective niche.

Finally, one may not be able to draw conclusions about the effects of hybridization on phenotypic variability without information on later generation hybrids. More insight can be gained from the average gene expression patterns reported in hybrids between incipient species of another salmonid: the dwarf and normal whitefish, *Coregonus clupeaformis*. In this system, F_1 hybrids gene expression mostly resembled the normal whitefish, and some genes were transgressive (which is comparable to our results), but transgressive expression prevailed in backcrosses (Renaut et al., 2009). Therefore, more extreme characters may be expected in later Arctic charr hybrids generations. However, the effects of hybridization on the hybrid phenotype also depend on many factors, like the genetic architecture and the selective regime (Albertson & Kocher, 2004). Similarly, underdominance in gene expression predominate in F_1 hybrids of brook charr, *Salvelinus fontinalis* (Mavarez, Audet, & Bernatchez, 2009), which is contrasting with our results and with those from Renaut and colleagues (Renaut et al., 2009). Overall, the consistent patterns of nonadditive inheritance of both average gene expression and gene expression variability suggest that post-zygotic reproductive isolation might already emerge among F_1 hybrids of PL- and SB-charr.

Materials and Methods.

Sampling.

We collected mature small SB- and PL-charr in Lake Thingvallavatn with gillnets. We generated 12 families, including 6 families of pure morph crosses (3 SBxSB; 3 PLxPL) and 6 families of reciprocal hybrids (maternal x paternal morph: 3 PLxSB; 3 PLxPL). The eggs were reared at approximately 5°C in a hatching tray (EWOS, Norway) under constant water flow and in complete darkness at the Holar University experimental facilities in Verið, Sauðárkrókur. Water temperature was recorded twice a day to estimate the relative age of the embryos in tau-somite units (τ_s), defined as the time to form one somite pair at a given temperature (Gorodilov, 1996). We sampled 9 embryos per family at two developmental time points ($150\tau_s$ and $200\tau_s$), summing 72 biological replicates. Samples were flash frozen in RNAlater (Ambion) and stored at -80°C. Prior to freezing, the eggs were permeabilized by a needle puncture. The samples were divided into 3 batches, each containing 3 embryos per family, to produce the sequencing libraries.

mRNA sequencing.

Total RNA was extracted using a standard Trisol protocol (samples quality: RIN 8.5-10). The samples were sent to BGI Europe (Copenhagen, Denmark) for mRNA enrichment, purification, fragmentation, adaptor ligation, PCR and sequencing on a DNBSEQ platform.

Small RNA sequencing.

Total RNA from the same samples as for the mRNA analyses was enriched for small RNAs using the mirVana kit (Ambion). The purity and amount of small RNA was verified on a BioAnalyzer (Agilent Technologies). The samples were prepared for sequencing following the small RNA v1.5 sample preparation protocol from Illumina. Briefly, 3' and 5' RNA adapters were ligated to small RNAs, which were subsequently, reverse transcribed into DNA and PCR amplified. The samples were then run on polyacrylamide gels and the DNA eluted from bands corresponding to 20-30 nucleotide RNA fragments. MiRNA sequencing (mRNA-seq) was performed at deCODE Genetics (Reykjavik, Iceland) using the TruSeq smallRNA (v1.5) kit (Illumina) on an Illumina GAIIX instrument.

Data pre-processing.

The sequencing data were pre-processed following the guidelines described Delhomme and colleagues (Delhomme et al., 2014). For mRNAs, adapter removal and filtering were done by the sequencing third party, but we re-assessed reads quality with FASTQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The reads were then aligned to the *Salvelinus sp*. genome (Christensen et al., 2018) using STAR (87; settings --outSAMstrandField intronMotif --twopassMode Basic) and were counted at the gene level using FeatureCounts (88; settings -p -B -C).

For miRNAs, we checked the quality of reads with FASTQC before and after removing the adapter sequences with Cutadapt (Martin, 2011). The miRNA transcripts were then quantified with MiRDeep2 (Friedländer, MacKowiak, Li, Chen, & Rajewsky, 2012) by pre-processing and mapping the reads to the *Salvelinus sp*. genome with the Mapping module before counting the number of precursor and mature sequences with the Quantifier module, using the MiRBase reference database for all species (Kozomara, Birgaoanu, & Griffiths-Jones, 2019). Because redundant miRNA homologs can be found across species, we removed sequences exhibiting at least 95% similarity across species using Cd-hit (Li & Godzik, 2006).

Gene expression variability.

Estimating gene expression variability is not straightforward because gene expression variance is dependent on the expression level. We applied the methods from Simonovsky et al. (2019) to calculate Local Coefficients of Variation (LCVs) at the gene level. Briefly, an algorithm uses a sliding window on genes ordered by expression level, ranks the Coefficient of Variation of the focal gene to that of the other genes located in the current window, and determines the percentile that fits the ranking of this coefficient of variation. Hence, LCV scores range from 0 (least variable genes) to 100 (most variable genes). We set the window size to 500 genes.

We assessed variations in LCV amongst cross types with ComplexHeatmap (Gu, Eils, & Schlesner, 2016). Gene clusters based on LCV covariation were extracted, and LCV scores were used as a response variable in linear models (one model per cluster) to estimate gene variability differences between cross types and the underlying inheritance pattern of the genes constituting each cluster. We fitted the models using MCMCglmm (Hadfield, 2010), specifying weakly informative priors (V=1, nu = 0.002) and determining the quality of the output from trace plots and posterior density plots. We set the number of iterations, thinning interval and burnin to 13000, 10, and 3000, respectively, for mRNAs, and to 130000, 1000,

and 3000 for miRNAs. Inferences were made based on the posterior modes and the overlaps in 95% Credible Intervals. Note that *variation* and *variability* were not used interchangeably through the paper. We followed Hallgrímsson et al. (2002), referring to variability as the *tendency* of a system (e.g., an organism) to vary.

Average gene expression.

We used DESeq2 (Love, Huber, & Anders, 2014) to estimate differential expression between cross types for both mRNAs and miRNAs. We corrected for false discovery by applying $\log_2$ fold change shrinkage with the `ashr` function from Stephens (2017). Overall dominance in gene expression was estimated by testing for differences in the proportion of differentially expressed genes between reciprocal hybrids and pure morph crosses. We then identified candidate genes with putative dominance in expression with handwritten *R* functions, according to the rationale described in Table S10.

Functional analyses.

We inferred the mRMA targets of candidate miRNAs with miRanda (Enright et al., 2003). Predictions were made using the mature miRNA sequences and the 3´ Untranslated Region (UTR) of the mRNA transcripts with more than 10 reads in our count datasets. The 3´ UTRs were retrieved from the *Salvelinus sp.* genome (Christensen et al., 2018). We run miRanda with default parameters and filtered the output by keeping the 10 targets of each miRNA with the highest total score.

Finally, we conducted gene ontology (GO) analyses of genes from clusters exhibiting different expression variability, differential average expression, or being identified as target genes of miRNAs of interests. We performed the enrichment analyses with the topGO R package (Alexa, Rahnenführer, & Lengauer, 2006), using the `weight01` algorithm and making statistical inferences based on Fisher's exact test. The GO annotations were retrieved from the *Salvelinus sp.* genome repository (Christensen et al., 2018).

Phenotyping and analyses of jaw morphology.

We studied the internal jaw morphology SBxsSB, PLxPL and SBxPL embryos produced form wild specimens in 2017. The embryos were reared in the same hatching tray as described above, sampled at ca. $460\tau_s$, and stained in one batch for bone (alizarin red) and cartilage (alcian blue), following the protocol from Kapralova et al. (2015). We photographed the specimens in lateral view, pinned down with needles in a Petri dish of 1.5% agarose gel and 10.5 ml of 80% glycerol. The photos were taken with a Leica microscope (M125), magnification 8X. 495 specimens exhibited a sufficiently mineralised low jaw for precise linear measurements (Table 4).

We measured the length and the angle of out-lever arm and the in-lever arm for jaw opening (Fig. 6a) on the photographs using ImageJ (Schneider, Rasband, & Eliceiri, 2012). Variations among cross types in the jaw lever system were estimated by fitting two Generalized Linear Mixed Models in MCMCglmm (Hadfield, 2010), using *I/O* ratio or α angle in each as predictor. We set the egg clutch identity (*i.e.* family) as a random factor, specified weakly informative priors (V=1, nu = 0.002), 650000 iterations, a 500 thinning

interval and 150000 burnins. Again, we determined the quality of the model estimates by examining the effective sample sizes, trace plots and posterior density plots.

Ethics statement.

Sampling of wild fish was conducted by the authors with the permission of the Thingvellir National Park Commission and the owner of the Mjóanes farm. ZOJ and KHK hold special permits for sampling fish for scientific purposes according to Icelandic law (clause 26 of law 61/2006 on salmonid fishing). After being stripped for gametes, parent fish were killed by a sharp blow to the head and checked for absence of breathing when placed in water. Setting up crosses and the subsequent killing of parents was performed by the authors. Ethics committee approval is not needed for scientific fishing in Iceland (The Icelandic law on animal protection, Law 15/1994, last updated with Law 157/2012). Rearing of embryos was performed according to Icelandic regulations (licence granted to Hólar University College aquaculture and experimental facilities). Sampling of embryos was performed by KHK. HUC-ARC has an operational license according to Icelandic law on aquaculture (Law 71/2008), that includes clauses of best practices for animal care and experiments.

Acknowledgments

We are very thankful to Benedikt Hallgrímsson for this help in developing the conceptual significance of the study. We thank Sigurður S. Snorrason, Skúli Skúlason, Neil Metcalfe, Michael Morrissey, and Arnar Pálsson for their theoretical guidance. We also thank Sébastien L. T. Matlosz, Lea J. Plesec, Marina de la Cámara, Jóhannes Guðbrandsson, and Ingeborg J. Klarenberg for their help in processing and analysing the data. We are grateful to Bjarni K. Kristjánsson, Kári H. Árnason, Rakel Þorbjörnsdóttir, and Christian Beuvard for the organisation and the maintenance of the experimental setup. Field work was conducted with help from the Arctic Charr and Salmonids Group members (University of Iceland) and the farmers of Mjóanes, Jóhann Jónsson and Rósa Jónsdóttir.

Author Contributions

Conceived the experiments: KHK and ZOJ. Molecular work: KHK. Collection of wild specimens and embryo crossing: KHK, ZOJ, LP, DAR and QJH. Embryo rearing: KHK and QJH. Embryo staining and collection of phenotypic data: LP. Data analyses: QJH and DAR. Manuscript writing: QJH, KHK, ZOJ, DAR and LP.

The authors declare having no conflict of interest.

References.

Abzhanov A, Protas M, Grant BR, Grant PR, Tabin CJ. 2004. Bmp4 and morphological variation of beaks in Darwin's finches. *Science.* 305:1462–1465.

Ackermann RR, Rogers J, Cheverud JM. 2006. Identifying the morphological signatures of hybridization in primate and human evolution. *J. Hum. Evol.* 51:632–645.

Adams DC, Nistri A. 2010. Ontogenetic convergence and evolution of foot morphology in European cave salamanders (Family: Plethodontidae). *BMC Evol. Biol.* 10.

Albertson C, Kocher T. 2004. Genetic architecture sets limits on transgressive segregation in hybrid cichlid fishes. *Evolution (N. Y).* 59:686–690.

Albertson RC, Streelman JT, Kocher TD. 2003. Directional selection has shaped the oral jaws of Lake Malawi cichlid fishes. *Proc. Natl. Acad. Sci.* [Internet] 100:5252–5257. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.0930235100

Albertson RC, Streelman JT, Kocher TD, Yelick PC. 2005. Integration and evolution of the cichlid mandible: The molecular basis of alternate feeding strategies. *Proc. Natl. Acad. Sci. U. S. A.* 102:16287–16292.

Alexa A, Rahnenführer J, Lengauer T. 2006. Improved scoring of functional groups from gene expression data by decorrelating GO graph structure. *Bioinformatics* 22:1600–1607.

Alibert P, Renaud S, Dod B, Bonhomme F, Auffray JC. 1994. Fluctuating asymmetry in the *Mus musculus* hybrid zone: A heterotic effect in disrupted co-adapted genomes. *Proc. R. Soc. B Biol. Sci.* 258:53–59.

Ason B, Darnell DK, Wittbrodt B, Berezikov E, Kloosterman WP, Wittbrodt J, Antin PB, Plasterk RHA. 2006. Differences in vertebrate microRNA expression. *Proc. Natl. Acad. Sci.* [Internet] 103:14385–14389. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.0603529103

Beck S V, Räsänen K, Ahi EP, Kristjánsson BK, Skúlason S, Jónsson ZO, Leblanc CA. 2019. Gene expression in the phenotypically plastic Arctic charr (*Salvelinus alpinus*): A focus on growth and ossification at early stages of development. *Evol. Dev.* [Internet] 21:16–30. Available from: https://onlinelibrary.wiley.com/doi/10.1111/ede.12275

Bhullar B-AS, Marugán-Lobón J, Racimo F, Bever GS, Rowe TB, Norell MA, Abzhanov A. 2012. Birds have paedomorphic dinosaur skulls. *Nature* [Internet] 487:223–226. Available from: http://www.nature.com/doifinder/10.1038/nature11146

Bicskei B, Taggart JB, Bron JE, Glover KA. 2020. Transcriptomic comparison of communally reared wild, domesticated and hybrid Atlantic salmon fry under stress and control conditions. *BMC Genet.* 21:1–20.

Bougas B, Granier S, Audet C, Bernatchez L. 2010. The transcriptional landscape of cross-specific hybrids and its possible link with growth in brook charr (*Salvelinus fontinalis*

Mitchill). *Genetics* 186:97–107.

Brachmann MK, Parsons K, Skúlason S, Ferguson MM. 2021. The interaction of resource use and gene flow on the phenotypic divergence of benthic and pelagic morphs of Icelandic Arctic charr (*Salvelinus alpinus*). *Ecol. Evol.*:1–20.

Christensen K, Rondeau E, Minkley D, Leong J, Nugent C, Danzmann R, Ferguson M, Stadnik A, Devlin R, Davidson W, et al. 2018. The Arctic charr (*Salvelinus alpinus*) genome and transcriptome. :1–30.

Coolon JD, Mcmanus CJ, Stevenson KR, Coolon JD, Mcmanus CJ, Stevenson KR, Graveley BR, Wittkopp PJ. 2014. Tempo and mode of regulatory evolution in *Drosophila*. *Genome Res.* 24:797–808.

Cooper WJ, Parsons K, McIntyre A, Kern B, McGee-Moore A, Albertson RC. 2010. Bentho-pelagic divergence of cichlid feeding architecture was prodigious and consistent during multiple adaptive radiations within African Rift-Lakes. *PLoS One* 5.

Coyne JA, Orr AH. 2004. Speciation. 1st ed. Sunderland, Massachusetts: Sinauer Associates, Inc.

Currey MC, Bassham S, Perry S, Cresko WA. 2017. Developmental timing differences underlie armor loss across threespine stickleback populations. *Evol. Dev.* 19:231–243.

Dagilis AJ, Kirkpatrick M, Bolnick DI. 2019. The evolution of hybrid fitness during speciation. *PLoS Genet.* 15:1–21.

Delhomme N, Mähler N, Schiffthaler B, Sundell D, Mannapperuma C, Hvidsten T, Street NR. 2014. Guidelines for RNA-Seq data analysis. *Epigenesys Protoc.* 67:1–24.

Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. 2013. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29:15–21.

Doenz CJ, Krähenbühl AK, Walker J, Seehausen O, Brodersen J. 2019. Ecological opportunity shapes a large Arctic charr species radiation. *Proc. R. Soc. B Biol. Sci.* 286.

Enright AJ, John B, Gaul U, Tuschl T, Sander C, Marks DS. 2003. MicroRNA targets in *Drosophila*. *Genome Biol.* 5.

Evgeny E V., Markev, Grigorii N, Pichugin MY. 2018. Juvenile divergence in adaptive traits among seven sympatric fish eco-morphs arises before moving to different lacustrine habitats. *J. Evol. Biol.*:1–56.

Friedländer MR, MacKowiak SD, Li N, Chen W, Rajewsky N. 2012. MiRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. *Nucleic Acids Res.* 40:37–52.

Gibson G, Weir B. 2005. The quantitative genetics of transcription. *Trends Genet.* 21:616–623.

Giraldez AJ, Cinalli RM, Glasner ME, Enright AJ, Thomson JM, Baskerville S, Hammond SM, Bartel DP, Schier AF. 2005. MicroRNAs regulate brain morphogenesis in zebrafish. *Science (80-.).* 308:833–838.

Gorodilov YN. 1996. Description of the early ontogeny of the Atlantic salmon, *Salmo salar*, with a novel system of interval (state) identification Yuriy. *Environ. Biol. Fishes* 47:109–127.

Green RM, Fish JL, Young NM, Smith FJ, Roberts B, Dolan K, Choi I, Leach CL, Gordon P, Cheverud JM, et al. 2017. Developmental nonlinearity drives phenotypic robustness. *Nat. Commun.* [Internet] 8. Available from: http://dx.doi.org/10.1038/s41467-017-02037-7

Green RM, Leach CL, Diewert VM, Aponte JD, Schmidt EJ, Cheverud JM, Roseman CC, Young NM, Marcucio RS, Hallgrímsson B. 2019. Nonlinear gene expression-phenotype relationships contribute to variation and clefting in the A/WySn mouse. *Dev. Dyn.* 248:1232–1242.

Gu Z, Eils R, Schlesner M. 2016. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* 32:2847–2849.

Guðbrandsson J, Kapralova KH, Franzdóttir SR, Bergsveinsdóttir TM, Hafstað V, Jónsson ZO, Snorrason SS, Pálsson A. 2019. Extensive genetic divergence between recently evolved sympatric Arctic charr morphs. *Ecol. Evol.* [Internet] 9:1–20. Available from: http://biorxiv.org/content/early/2018/12/07/489104.abstract

Hadfield JD. 2010. MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. *J. Stat. Softw.* 33:1–22.

Hallgrimsson B, Green RM, Katz DC, Fish JL, Bernier FP, Roseman CC, Young NM, Cheverud JM, Marcucio RS. 2019. The developmental-genetics of canalization. *Semin. Cell Dev. Biol.* [Internet] 88:67–79. Available from: https://doi.org/10.1016/j.semcdb.2018.05.019

Hallgrímsson B, Willmore K, Hall BK. 2002. Canalization, developmental stability, and morphological integration in primate limbs. *Yearb. Phys. Anthropol.* 45:131–158.

Horta-Lacueva QJB, Benhaïm D, Morrissey MB, Snorrason SS, Kapralova KH. 2020. Animal personality adds complexity to the processes of adaptive divergence and speciation. *Anim. Behav.* [Internet] 175:57–73. Available from: https://doi.org/10.1016/j.anbehav.2021.02.022

Horta-Lacueva QJB, Snorrason SS, Morrissey MB, Leblanc CA, Kapralova KH. 2021. Multivariate analysis of morphology, behaviour, growth and developmental timing in hybrids brings new insights into the divergence of sympatric Arctic charr morphs. *BMC Ecol. Evol.* [Internet] 21:1–15. Available from: https://doi.org/10.1101/2020.10.14.339911

Ji X, Jiang P, Luo J, Li M, Bai Y, Zhang J, Han B. 2020. Identification and characterization of miRNAs involved in cold acclimation of zebrafish ZF4 cells.Cui Z, editor. *PLoS One* [Internet] 15:e0226905. Available from:

https://dx.plos.org/10.1371/journal.pone.0226905

Jonsson B, Skúlason S, Snorrason SS, Sandlund OT, Malmquist HJ, Jónasson PM, Gydemo R, Lindem T. 1988. Life history variation of polymorphic Arctic charr (*Salvelinus alpinus*) in Thingvallavatn, Iceland. *Can. J. Fish. Aquat. Sci.* 45:1537-1547.

Kapralova KH, Franzdóttir SR, Jónsson H, Snorrason SS, Jónsson ZO. 2014. Patterns of MiRNA expression in Arctic charr development. *PLoS One* 9.

Kapralova KH, Guðbrandsson J, Reynisdottir S, Santos CB, Baltanás VC, Maier VH, Snorrason SS, Palsson A. 2013. Differentiation at the MHCIIα and Cath2 Loci in Sympatric *Salvelinus alpinus* resource morphs in Lake Thingvallavatn. *PLoS One* 8.

Kapralova KH, Jónsson ZO, Palsson A, Franzdóttir SR, le Deuff S, Kristjánsson BK, Snorrason SS. 2015. Bones in motion: Ontogeny of craniofacial development in sympatric Arctic charr morphs. *Dev. Dyn.* 244:1168–1178.

Kapralova KH, Morrissey MB, Kristjánsson BK, Olafsdóttir GÁ, Snorrason SS, Ferguson MM. 2011. Evolution of adaptive diversity and genetic connectivity in Arctic charr (*Salvelinus alpinus*) in Iceland. *Heredity (Edinb).* [Internet] 106:472–487. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3131972&tool=pmcentrez&rendertype=abstract

Kapsimali M, Kloosterman WP, de Bruijn E, Rosa F, Plasterk RHA, Wilson SW. 2007. MicroRNAs show a wide diversity of expression profiles in the developing and mature central nervous system. *Genome Biol.* 8.

Klemetsen A. 2010. The Charr Problem Revisited: Exceptional Phenotypic Plasticity Promotes Ecological Speciation in Postglacial Lakes. *Freshw. Rev.* 3:49–74.

Knudsen R, Klemetsen A, Amundsen P-A, Hermansen B. 2006. Incipient speciation through niche expansion: an example from the Arctic charr in a subarctic lake. *Proc. R. Soc. B Biol. Sci.* [Internet] 273:2291–2298. Available from: http://rspb.royalsocietypublishing.org/cgi/doi/10.1098/rspb.2006.3582

Kozomara A, Birgaoanu M, Griffiths-Jones S. 2019. MiRBase: From microRNA sequences to function. *Nucleic Acids Res.* 47:D155–D162.

Küttner E, Parsons KJ, Robinson BW, Skúlason S, Danzmann RG, Ferguson MM. 2013. Effects of population, family, and diet on craniofacial morphology of Icelandic Arctic charr (*Salvelinus alpinus*). *Biol. J. Linn. Soc.* [Internet] 108:702–714. Available from: https://academic.oup.com/biolinnean/article-lookup/doi/10.1111/j.1095-8312.2012.02038.x

De la Cámara M, Ponsioen Lieke, Horta-Lacueva QJB, Kapralova KH. 2021. The dynamic ontogenetic patterns of adaptive divergence and sexual and dimorphism in Arctic charr. *bioRxiv* [Internet]:2021.01.15.426104. Available from: https://doi.org/10.1101/2021.01.15.426104

Landry CR, Hartl DL, Ranz JM. 2007. Genome clashes in hybrids: Insights from gene

expression. *Heredity (Edinb).* 99:483–493.

Lazić MM, Carretero MA, Crnobrnja-Isailović J, Kaliontzopoulou A. 2014. Effects of Environmental Disturbance on Phenotypic Variation: An Integrated Assessment of Canalization, Developmental Stability, Modularity, and Allometry in Lizard Head Shape. *Am. Nat.* 185:44–58.

Li W, Godzik A. 2006. Cd-hit: A fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* 22:1658–1659.

Liao Y, Smyth GK, Shi W. 2014. FeatureCounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* 30:923–930.

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15:1–21.

Ma Y, Zang L, Wang D, Jiang J, Wang C, Wang X, Fang F, Wang H. 2019. Effects of miR-181a-5p abnormal expression on zebrafish (*Danio rerio*) vascular development following triclosan exposure. *Chemosphere* [Internet] 223:523–535. Available from: https://doi.org/10.1016/j.chemosphere.2019.02.038

Mack KL, Nachman MW. 2017. Gene Regulation and Speciation. *Trends Genet.* [Internet] 33:68–80. Available from: http://dx.doi.org/10.1016/j.tig.2016.11.003

Malmquist AHJ, Snorrason SS, Skúlason S, Jonsson B, Sandlund OT, Jonasson M. 1992. Diet Differentiation in Polymorphic Arctic Charr in Thingvallavatn , Iceland. *J. Anim. Ecol.* 61:21–35.

Mar JC. 2019. The rise of the distributions: why non-normality is important for understanding the transcriptome and beyond. *Biophys. Rev.* 11:89–94.

Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. J.* 10:10–12.

Mavarez J, Audet C, Bernatchez L. 2009. Major disruption of gene expression in hybrids between young sympatric anadromous and resident populations of brook charr (*Salvelinus fontinalis* Mitchill). *J. Evol. Biol.* 22:1708–1720.

Nakamura K, Maki N, Trinh A, Trask HW, Gui J, Tomlinson CR, Tsonis PA. 2010. MiRNAs in newt lens regeneration: Specific control of proliferation and evidence for miRNA networking. *PLoS One* 5:1–7.

Østbye K, Hagen Hassve M, Peris Tamayo AM, Hagenlund M, Vogler T, Præbel K. 2020. “And if you gaze long into an abyss, the abyss gazes also into thee”: four morphs of Arctic charr adapting to a depth gradient in Lake Tinnsjøen. *Evol. Appl.*:1240–1261.

Parsons KJ, Concannon M, Navon D, Wang J, Ea I, Groveas K, Campbell C, Albertson RC. 2016. Foraging environment determines the genetic architecture and evolutionary potential of trophic morphology in cichlid fishes. *Mol. Ecol.* 25:6012–6023.

Parsons KJ, Sheets HD, Skúlason S, Ferguson MM. 2011. Phenotypic plasticity,

heterochrony and ontogenetic repatterning during juvenile development of divergent arctic charr (*Salvelinus alpinus*). *J. Evol. Biol.* 24:1640–1652.

Parsons KJ, Skúlason S, Ferguson M. 2010. Morphological variation over ontogeny and environments in resource polymorphic arctic charr (*Salvelinus alpinus*). *Evol. Dev.* 12:246–257.

Parsons KJ, Trent Taylor A, Powder KE, Albertson RC. 2014. Wnt signalling underlies the evolution of new phenotypes and craniofacial variability in Lake Malawi cichlids. *Nat. Commun.* [Internet] 5:1–11. Available from: http://dx.doi.org/10.1038/ncomms4629

Pavey SA, Collin H, Nosil P, Rogers SM. 2010. The role of gene expression in ecological speciation. *Ann. N. Y. Acad. Sci.* 1206:110–129.

Pélabon C, Carlson ML, Hansen TF, Yoccoz NG, Armbruster WS. 2004. Consequences of inter-population crosses on developmental stability and canalization of floral traits in *Dalechampia scandens* (Euphorbiaceae). *J. Evol. Biol.* 17:19–32.

Pesevski M, Dworkin I. 2020. Genetic and environmental canalization are not associated among altitudinally varying populations of *Drosophila melanogaster*. *Evolution (N. Y).* 74:1755–1771.

Pfennig DW, Martin RA. 2009. A maternal effect mediates rapid population divergence and character displacement in spadefoot toads. *Evolution (N. Y).* 63:898–909.

Pichugin MY. 2009. The development of an artificial hybrid and revealing elements of reproductive isolation between sympatric forms of Dryagin's char and *Salvelinus alpinus* complex (Salmonidae) from Sobachye Mountain Lake (Taimyr). *J. Ichthyol.* 49:236–248.

Renaut S, Nolte AW, Bernatchez L. 2009. Gene Expression Divergence and Hybrid Misexpression between Lake Whitefish Species Pairs (*Coregonus spp*. Salmonidae). *Mol. Biol. Evol.* [Internet] 26:925–936. Available from: https://academic.oup.com/mbe/article-lookup/doi/10.1093/molbev/msp017

Roberts RB, Hu Y, Albertson RC, Kocher TD. 2011. Craniofacial divergence and ongoing adaptation via the hedgehog pathway. *Proc. Natl. Acad. Sci.* [Internet] 108:13194–13199. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.1018456108

Sandlund OT, Gunnarsson K, Jónasson PM, Jonsson B, Lindem T, Magnússon KP, Malmquist HJ, Sigurjónsdóttir H, Skúlason S, Snorrason SS. 1992. The Arctic charr *Salvelinus alpinus* in Thingvallavatn. *Oikos* 64:305–351.

Santos-Santos JH, Audenaert L, Verheyen E, Adriaens D. 2021. Ontogenetic divergence generates novel phenotypes in hybrid cichlids. *J. Anat.* [Internet] 238:1116–1127. Available from: https://onlinelibrary.wiley.com/doi/10.1111/joa.13375

Satokangas I, Martin SH, Helanterä J, J. K. 2020. Multi-locus interactions and the build-up of reproductive isolation. *Philos. Trans. R. Soc. B-Biological Sci.* 375.

Schneider CA, Rasband W, Eliceiri K. 2012. ImageJ. *Nat. Methods* 9:671–675.

Selz OM, Lucek K, Young KA, Seehausen O. 2014. Relaxed trait covariance in interspecific cichlid hybrids predicts morphological diversity in adaptive radiations. *J. Evol. Biol.* [Internet] 27:11–24. Available from: https://onlinelibrary.wiley.com/doi/10.1111/jeb.12283

Selz OM, Thommen R, Pierotti MER, Anaya-Rojas JM, Seehausen O. 2016. Differences in male coloration are predicted by divergent sexual selection between populations of a cichlid fish. *Proc. R. Soc. B Biol. Sci.* [Internet] 283:20160172. Available from: http://rspb.royalsocietypublishing.org/lookup/doi/10.1098/rspb.2016.0172

Shi J, Li T, Chen L, Aihara K. 2019. Quantifying pluripotency landscape of cell differentiation from scRNA-seq data by continuous birth-death process. *PLoS Comput. Biol.* [Internet] 15:1–17. Available from: http://dx.doi.org/10.1371/journal.pcbi.1007488

Siciliano V, Garzilli I, Fracassi C, Criscuolo S, Ventre S, Di Bernardo D. 2013. MiRNAs confer phenotypic robustness to gene networks by suppressing biological noise. *Nat. Commun.* 4.

Simonovsky E, Schuster R, Yeger-Lotem E. 2019. Large-scale analysis of human gene expression variability associates highly variable drug targets with lower drug effectiveness and safety. *Bioinformatics* [Internet] 35:3028–3037. Available from: https://academic.oup.com/bioinformatics/article/35/17/3028/5289324

Skúlason S, Parsons KJ, Svanbäck R, Räsänen K, Ferguson MM, Adams CE, Amundsen P, Bartels P, Bean CW, Boughman JW, et al. 2019. A way forward with eco evo devo: an extended theory of resource polymorphism with postglacial fishes as model systems. *Biol. Rev.* [Internet] 94:1786–1808. Available from: https://onlinelibrary.wiley.com/doi/10.1111/brv.12534

Skúlason S, Snorrason SS, Noakes DLG, Ferguson MM, Malmquist HJ. 1989. Segregation in spawning and early life history among polymorphic Arctic charr, *Salvelinus alpinus*, in Thingvallavatn, Iceland. *J. Fish Biol.* 35:225–232.

Skúlason S, Snorrason SS, Ota D, Noakes DLG. 1993. Genetically based differences in foraging behaviour among sympatric morphs of Arctic charr (Pisces: Salmonidae). *Anim. Behav.* 45:1179–1192.

Snorrason SS, Skulason S, Jonsson B, Malmquist HJ, Jonasson PM, Sandlund OT, Lindem T. 1994. Trophic specialization in Arctic charr *Salvelinus alpinus* (Pisces; Salmonidae): morpholigcal divergence and ontogenic niche shifts. *Biol. J. Linn. Soc.* 52:1–18.

Soni K, Choudhary A, Patowary A, Singh AR, Bhatia S, Sivasubbu S, Chandrasekaran S, Pillai B. 2013. MiR-34 is maternally inherited in *Drosophila melanogaster* and *Danio rerio*. *Nucleic Acids Res.* 41:4470–4480.

Stahlhut C, Suárez Y, Lu J, Mishima Y, Giraldez AJ. 2012. miR-1 and miR-206 regulate angiogenesis by modulating VegfA expression in zebrafish. *Dev.* 139:4356–4364.

Stelkens RB, Schmid C, Selz O, Seehausen O. 2009. Phenotypic novelty in experimental hybrids is predicted by the genetic distance between species of cichlid fish. *BMC Evol. Biol.* [Internet] 9:283. Available from: http://bmcevolbiol.biomedcentral.com/articles/10.1186/1471-2148-9-283

Stephens M. 2017. False discovery rates: A new deal. *Biostatistics* 18:275–294.

Swingler TE, Wheeler G, Carmont V, Elliott HR, Barter MJ, Abu-Elmagd M, Donell ST, Boot-Handford RP, Hajihosseini MK, Münsterberg A, et al. 2012. The expression and function of microRNAs in chondrogenesis and osteoarthritis. *Arthritis Rheum.* 64:1909–1919.

Teschendorff AE, Feinberg AP. 2021. Statistical mechanics meets single-cell biology. *Nat. Rev. Genet.* [Internet] 22:459–476. Available from: http://dx.doi.org/10.1038/s41576-021-00341-z

Thompson KA, Osmond MM, Schluter D. 2019. Parallel genetic evolution and speciation from standing variation. *Evol. Lett.* 3:129–141.

Thompson KA, Urquhart-Cronish M, Whitney KD, Rieseberg LH, Schluter D. 2020. Patterns, predictors, and consequences of dominance in hybrids. *Am. Nat.*

Thompson KA, Urquhart-Cronish M, Whitney KD, Rieseberg LH, Schluter D. 2021. Patterns, predictors, and consequences of dominance in hybrids. *Am. Nat.* [Internet] 197:E72–E88. Available from: https://www.journals.uchicago.edu/doi/10.1086/712603

Waddington CH. 1942. Canalization of development and the inheritance of acquired characters. *Nature* 150:563–565.

Wagner GP, Booth G, Bagheri-Chaichian H. 1997. A population genetic theory of canalization. *Evolution (N. Y).* 51:329–347.

Wainwright PC, Richard BA. 1995. Predicting patterns of prey use from morphology of fishes. *Environ. Biol. Fishes* 44:97–113.

Wienholds E, Kloosterman WP, Miska E, Alvarez-Saavedra E, Berezikov E, de Bruijn E, Horvitz HR, Kauppinen S, Plasterk RH a. 2005. MicroRNA expression in zebrafish embryonic development. *Science* [Internet] 309:310–311. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15919954

Zhuang S, Fu Y, Li J, Li M, Hu X, Zhu J, Tong M. 2020. MicroRNA-375 overexpression disrupts cardiac development of Zebrafish (*Danio rerio*) by targeting *notch2*. *Protoplasma* 257:1309–1318.

Supplementary figures and tables.

Table 1. Number and proportion of differentially expressed mRNA transcripts in each contrast, χ^2 and P-values contrast comparisons.

RNA	Contrast 1	Contrast 2	χ^2	*P*
mRNA	PLxSB/PLxPL 150τ_s = 13 (0.03%)	SBxPL/PLxPL 150τ_s = 14 (0.03%)	0.00	1.00
	PLxSB/SBxSB 150τ_s = 44 (0.11%)	SBxPL/SBxSB 150τ_s = 33 (0.08%)	1.29	0.25
	PLxSB/PLxPL 200τ_s = 337 (0.82%)	SBxPL/PLxPL 200τ_s = 192 (0.46%)	39.44	<0.01*
	PLxSB/SBxSB 200τ_s = 2504 (6.39%)	SBxPL/SBxSB 200τ_s = 82 (0.2%)	2339.08	<0.01*

* Significant after Bonferroni correction.

Table 2. Differentially expressed miRNAs between pure morph crosses, and miRNAs with nonadditive inheritance. Putative location/functions according to the literature.

	miRNA family (homologs)[1]	**Putative location/function[2]**
DE at 150τ_s	miR-100 (43), miR-148 (2), miR-181* (47), miR-199* (24), miR-375 (14), miR-455 (3)	Epithelia of pharyngeal arches, head skeleton and pectoral fins, epidermis of head, tip of the tail, brain, spinal cord thymic primordium, eyes, sense organs, in zebrafish/medaka (Ason et al., 2006; Wienholds et al., 2005) Cell metabolism and viability in zebrafish (Ji et al., 2020) Lens pigments epithelial cell proliferation in in newt (Nakamura et al., 2010) Cardiac development in zebrafish (Zhuang et al., 2020) Vascular development, zebrafish (Ma et al., 2019) Involved in chondrogenesis, human (Swingler et al., 2012)
DE at 200τ_s	let-7* (5), miR-1* (37), miR-10* (42), miR-124* (51), miR-125 (2), miR-128 (27), miR-132 (11), miR-138 (27), miR-148 (2), miR-181* (47), miR-193 (14), miR-199* (4), miR-20 (1), miR-200 (2), miR-203 (19), miR-206* (16), miR-2188* (5), miR-221 (1), miR-222 (20), miR-2478 (1), miR-27 (3), miR-30* (23), miR-301 (3), miR-429 (18), miR-430* (1), miR-455* (7), miR-725 (3), miR-737* (1), miR-9* (6), miR-92 (57), miR-9226 (1)	Brain, sense organs, eyes, spinal cord, skeletal muscles, gills, excretory/digestive system, pharyngeal arches, fins, epidermis of the head, tip of the tail, in zebrafish/medaka (Ason et al., 2006; Wienholds et al., 2005) Brain morphogenesis in zebrafish (Giraldez et al., 2005) Angiogenesis in muscles, in zebrafish (Stahlhut, Suárez, Lu, Mishima, & Giraldez, 2012)
Maternal at 200τ_s	miR-1* (25)	Body, head, fin muscles and skeletal muscles, in zebrafish/medaka (Wienholds et al. 2005; Ason et al. 2006)
PL-dominant at 150τ_s	miR-100 (43), miR-181 (47)	Brain; spinal cord, eyes, thymic primordium, sense organs, gills in zebrafish/medaka (Ason et al., 2006; Wienholds et al., 2005)

[1]Different miRNAs from the same family, the same miRNAs with different orientations, paralogs or putative orthologs (the reads have aligned to different sequences, from different species, but have the same miRNAs name)

*Also found to be differentially expressed between SB- and domesticated charr embryos by Kapralova and colleagues (Kalina H. Kapralova et al., 2014).

Table 3. LCV estimates of *ptch1* and *bmp4* at 200τ_s in each cross type.

Gene	PLxPL	PLxSB	SBxPL	SBxSB
ptch1	33.6	65.4	29.2	88.6
bmp4	No gene noise	No gene noise	No gene noise	No gene noise

Table 4. Linear measurement sample sizes per family[1], for each cross type.

	PLxPL	PLxSB	SBxSB
Family 1	36	77	73
Family 2	63	50	20
Family 3	-	92	-
Family 4	-	54	-
Family 5	-	30	-

[1]Full sibling design, no parents were used for more than one cross.

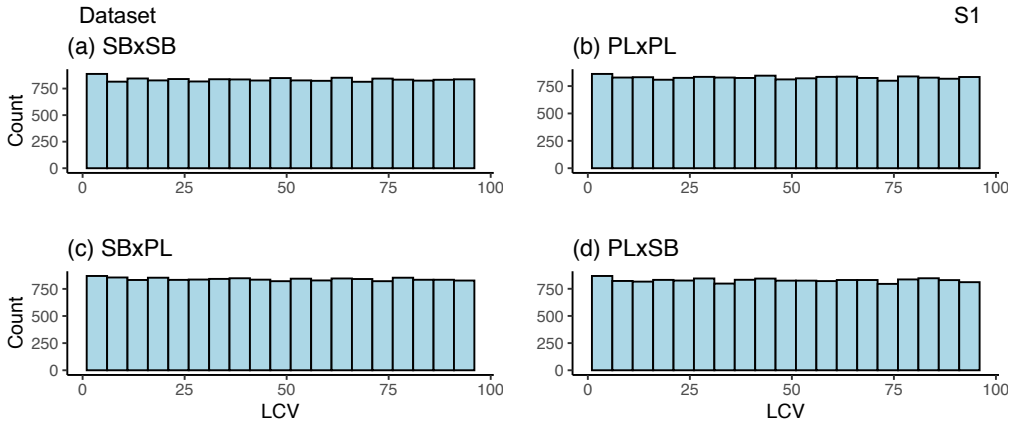

Fig. S1. Distribution of the local coefficents of variation (LCVs) of mRNAs expressed in each cross type, at $150\tau_s$.

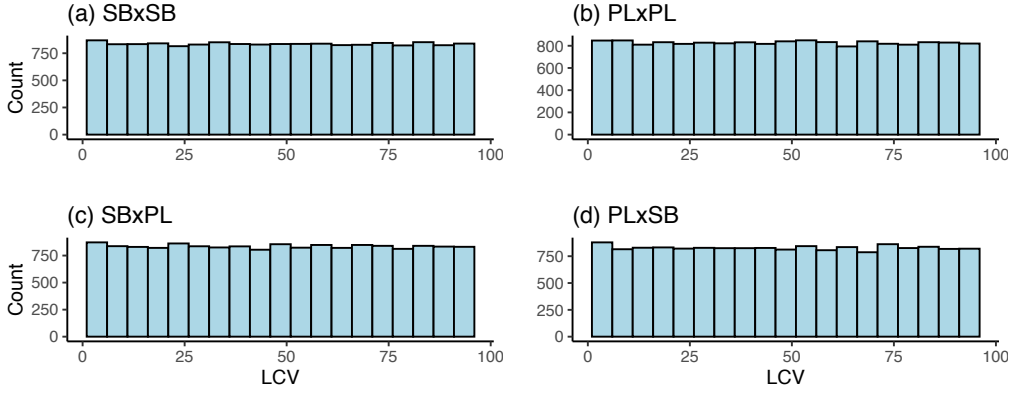

Fig. S2. Distribution of the local coefficents of variation (LCVs) of mRNAs expressed in each cross type, at $200\tau_s$.

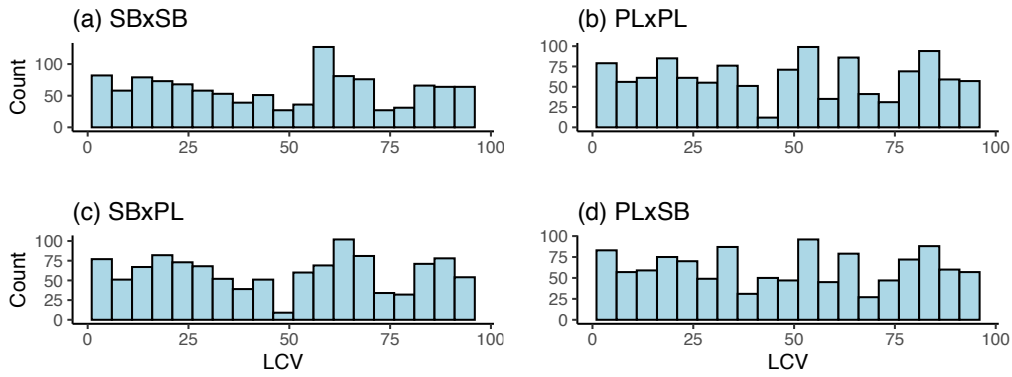

Fig. S3. Distribution of the local coefficents of variation (LCVs) of miRNAs expressed in each cross type, at $150\tau_s$.

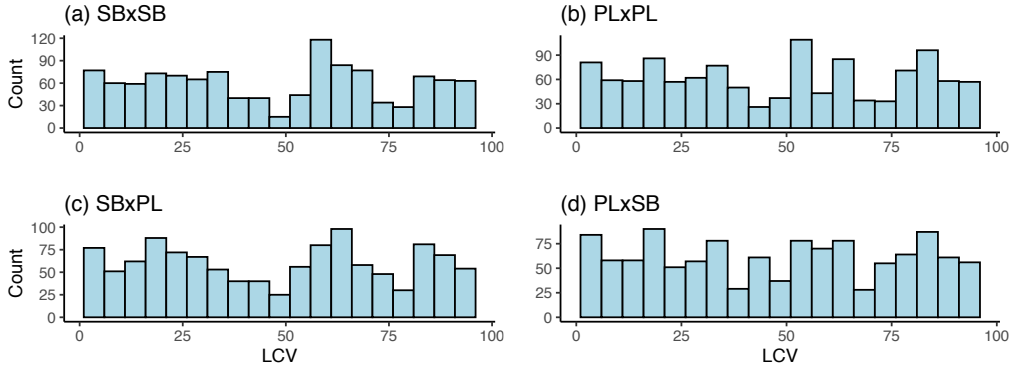

Fig. S4. Distribution of the local coefficents of variation (LCVs) of miRNAs expressed in each cross type, at $200\tau_s$.

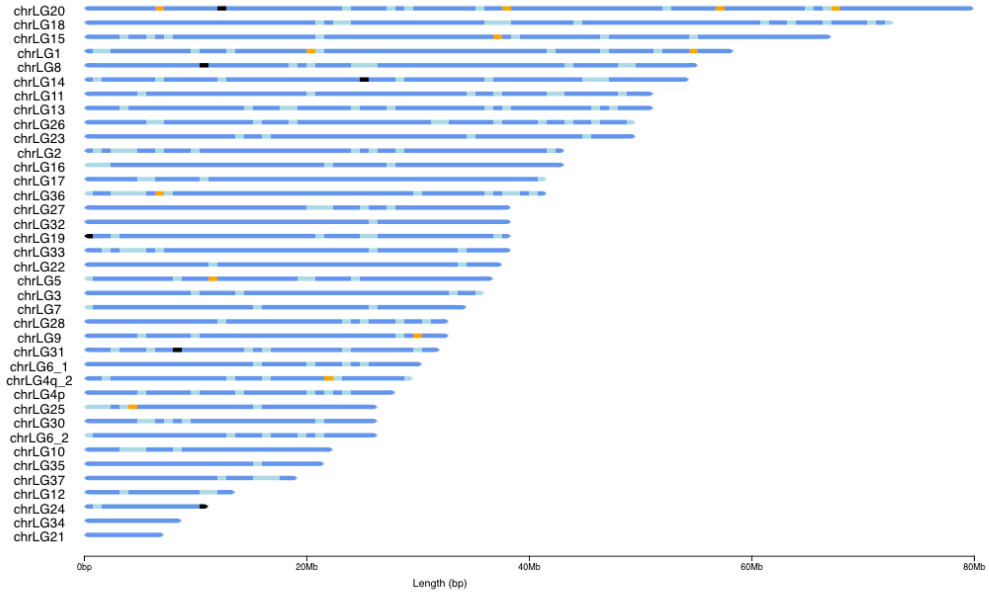

Fig. S5. Physical location of the genes with differential expression among the two pure morph crosses at $200\tau_s$. Blue: Differentially expressed with no putative dominance pattern. Light blue: No differential expression. Orange: Maternal inheritance, Black: PL-biased expression in hybrids. Uncharacterized chromosomes not shown.

Table S1. The first 10 GO terms associated with the mRNAs from each cluster of expression variability.

Cluster	GO ID	GO Term	Annotated genes	Significant genes	Expected	p-value
1	GO:0000398	mRNA splicing, via spliceosome	694	87	30.34	3.4e-08
	GO:0000381	regulation of alternative mRNA splicing,...	168	25	7.34	8.7e-08
	GO:0061077	chaperone-mediated protein folding	152	20	6.64	2.3e-07
	GO:0006368	transcription elongation from RNA polyme...	277	28	12.11	2.6e-07
	GO:0031053	primary miRNA processing	32	10	1.4	6.6e-07
	GO:0006458	'de novo' protein folding	101	13	4.42	1.3e-06
	GO:0042795	snRNA transcription by RNA polymerase II	117	18	5.11	3.3e-06
	GO:0006406	mRNA export from nucleus	228	29	9.97	6.3e-06
	GO:0000724	double-strand break repair via homologou...	179	21	7.83	6.5e-06
	GO:0006369	termination of RNA polymerase II transcr...	126	18	5.51	9.6e-06
2	GO:0003148	outflow tract septum morphogenesis	144	20	5.61	8.6e-07
	GO:0009948	anterior/posterior axis specification	412	36	16.06	1.8e-06
	GO:0072095	regulation of branch elongation involved...	17	7	0.66	1.9e-06
	GO:0006886	intracellular protein transport	2718	158	105.93	2.2e-06
	GO:0061055	myotome development	29	8	1.13	7.9e-06
	GO:0072193	ureter smooth muscle cell differentiatio...	40	9	1.56	1.8e-05
	GO:1904294	positive regulation of ERAD pathway	38	8	1.48	1.9e-05
	GO:0043282	pharyngeal muscle development	23	7	0.9	1.9e-05
	GO:0040037	negative regulation of fibroblast growth...	74	12	2.88	2.7e-05
	GO:0048538	thymus development	273	26	10.64	2.8e-05

3	GO:0051220	cytoplasmic sequestering of protein	81	10	3.26	1.8e-05
	GO:1902902	negative regulation of autophagosome ass...	39	9	1.57	1.9e-05
	GO:2000036	regulation of stem cell population maint...	123	11	4.96	1.9e-05
	GO:0002924	negative regulation of humoral immune re...	17	6	0.68	3.6e-05
	GO:0072310	glomerular epithelial cell development	98	7	3.95	3.7e-05
	GO:0060319	primitive erythrocyte differentiation	39	8	1.57	1.4e-04
	GO:0016032	viral process	1690	80	68.08	2.5e-04
	GO:0070125	mitochondrial translational elongation	104	13	4.19	2.8e-04
	GO:0070126	mitochondrial translational termination	105	13	4.23	3.1e-04
	GO:0031087	deadenylation-independent decapping of n...	16	5	0.64	3.2e-04
4	GO:0070125	mitochondrial translational elongation	104	26	4.31	8.9e-14
	GO:0070126	mitochondrial translational termination	105	25	4.35	8.8e-13
	GO:0010972	negative regulation of G2/M transition o...	220	30	9.12	1.8e-11
	GO:0061418	regulation of transcription from RNA pol...	173	26	7.17	8.4e-10
	GO:0002479	antigen processing and presentation of e...	134	24	5.55	1.4e-09
	GO:0043488	regulation of mRNA stability	431	43	17.86	6.1e-09
	GO:0008063	Toll signaling pathway	70	16	2.9	2.1e-08
	GO:0016579	protein deubiquitination	542	47	22.46	2.8e-08
	GO:0000398	mRNA splicing, via spliceosome	694	73	28.76	4.4e-08
	GO:0031146	SCF-dependent proteasomal ubiquitin-depe...	141	22	5.84	8.7e-08
5	GO:0002181	cytoplasmic translation	261	49	9.61	< 1e-30
	GO:0019083	viral transcription	301	45	11.08	2.7e-17
	GO:0006413	translational initiation	295	36	10.86	4.5e-16

	GO:0006614	SRP-dependent cotranslational protein ta...	69	21	2.54	7.3e-16
	GO:0000184	nuclear-transcribed mRNA catabolic proce...	145	27	5.34	3.5e-12
	GO:0006364	rRNA processing	404	47	14.88	1.3e-11
	GO:0000028	ribosomal small subunit assembly	48	14	1.77	1.2e-09
	GO:0006412	translation	1434	130	52.81	2.2e-07
	GO:1902306	negative regulation of sodium ion transm...	48	5	1.77	1.8e-06
	GO:0015031	protein transport	5299	233	195.13	5.5e-06
6	GO:0006120	mitochondrial electron transport, NADH t...	74	20	2.31	7.5e-14
	GO:0032981	mitochondrial respiratory chain complex ...	87	19	2.71	1.9e-11
	GO:0002181	cytoplasmic translation	261	27	8.14	3.9e-11
	GO:0019083	viral transcription	301	24	9.39	1.5e-08
	GO:0006414	translational elongation	207	17	6.46	1.7e-07
	GO:0009952	anterior/posterior pattern specification	1319	68	41.13	6.3e-07
	GO:0000184	nuclear-transcribed mRNA catabolic proce...	145	18	4.52	3.7e-06
	GO:0048653	anther development	12	5	0.37	1.9e-05
	GO:0010223	secondary shoot formation	12	5	0.37	1.9e-05
	GO:0006413	translational initiation	295	20	9.2	1.9e-05
7	GO:0002181	cytoplasmic translation	261	29	12.21	3.0e-07
	GO:0042407	cristae formation	52	12	2.43	3.8e-06
	GO:1902884	positive regulation of response to oxida...	53	8	2.48	1.1e-05
	GO:0001700	embryonic development via the syncytial ...	135	21	6.32	1.5e-05
	GO:0050821	protein stabilization	522	46	24.43	3.3e-05
	GO:0006886	intracellular protein transport	2718	170	127.18	3.6e-05
	GO:0043312	neutrophil degranulation	869	67	40.66	3.8e-05
	GO:1904693	midbrain morphogenesis	10	5	0.47	4.6e-05

	GO:0044804	autophagy of nucleus	32	8	1.5	8.7e-05
	GO:0006413	translational initiation	295	26	13.8	8.8e-05
8	GO:0043161	proteasome-mediated ubiquitin-dependent ...	1030	147	63.35	4.1e-15
	GO:0006886	intracellular protein transport	2718	281	167.16	9.7e-12
	GO:0006888	endoplasmic reticulum to Golgi vesicle-m...	438	72	26.94	1.6e-10
	GO:0036498	IRE1-mediated unfolded protein response	152	29	9.35	1.3e-09
	GO:0070936	protein K48-linked ubiquitination	168	36	10.33	5.5e-09
	GO:0015031	protein transport	5299	451	325.9	5.0e-08
	GO:0007032	endosome organization	203	37	12.49	7.8e-08
	GO:0035509	negative regulation of myosin-light-chai...	26	11	1.6	1.5e-07
	GO:0034067	protein localization to Golgi apparatus	88	21	5.41	1.5e-07
	GO:0018105	peptidyl-serine phosphorylation	1000	94	61.5	1.6e-07
9	GO:0060048	cardiac muscle contraction	697	96	40.88	3.5e-18
	GO:0046716	muscle cell cellular homeostasis	105	35	6.16	8.8e-18
	GO:0030049	muscle filament sliding	131	42	7.68	1.1e-12
	GO:0006942	regulation of striated muscle contractio...	498	66	29.21	2.7e-12
	GO:0003009	skeletal muscle contraction	169	39	9.91	7.7e-12
	GO:0045214	sarcomere organization	277	48	16.25	1.0e-11
	GO:0032201	telomere maintenance via semi-conservati...	30	15	1.76	2.1e-11
	GO:0000083	regulation of transcription involved in ...	82	21	4.81	5.2e-10
	GO:0071688	striated muscle myosin thick filament as...	65	17	3.81	8.5e-10
	GO:0055010	ventricular cardiac muscle tissue morpho...	306	50	17.95	9.9e-10
10	GO:0006406	mRNA export from nucleus	228	59	14.72	4.6e-20
	GO:0000398	mRNA splicing, via spliceosome	694	149	44.8	1.6e-17

GO:0060964	regulation of gene silencing by miRNA	236	42	15.23	1.2e-13
GO:0006409	tRNA export from nucleus	61	22	3.94	1.1e-11
GO:0045292	mRNA cis splicing, via spliceosome	95	32	6.13	3.1e-11
GO:0043488	regulation of mRNA stability	431	62	27.82	3.9e-10
GO:0031145	anaphase-promoting complex-dependent cat...	158	33	10.2	1.7e-09
GO:0006334	nucleosome assembly	149	38	9.62	4.8e-09
GO:0000245	spliceosomal complex assembly	148	38	9.55	1.1e-08
GO:0006271	DNA strand elongation involved in DNA re...	30	12	1.94	3.3e-08

Table S2. The first 10 GO terms of the putative targets of miRNAs from each cluster of expression variability.

Cluster	GO ID	GO Term	Annotated genes	Significant genes	Expected	p-value
1	GO:2001045	negative regulation of integrin-mediated...	20	2	0.05	1.2e-03
	GO:1900194	negative regulation of oocyte maturation	31	2	0.08	2.9e-03
	GO:0061386	closure of optic fissure	32	2	0.08	3.1e-03
	GO:0060235	lens induction in camera-type eye	33	2	0.09	3.3e-03
	GO:0021960	anterior commissure morphogenesis	39	2	0.1	4.6e-03
	GO:0070933	histone H4 deacetylation	39	2	0.1	4.6e-03
	GO:0032366	intracellular sterol transport	66	2	0.17	5.1e-03
	GO:1903206	negative regulation of hydrogen peroxide...	49	2	0.13	7.2e-03
	GO:0006820	anion transport	1649	7	4.28	1.0e-02
	GO:0060996	dendritic spine development	596	3	1.55	1.5e-02
2	GO:0051894	positive regulation of focal adhesion as...	130	3	0.25	1.9e-03

	GO:0060978	angiogenesis involved in coronary vascul...	45	2	0.09	3.3e-03
	GO:0050687	negative regulation of defense response ...	73	3	0.14	3.9e-03
	GO:0022408	negative regulation of cell-cell adhesio...	685	5	1.3	4.1e-03
	GO:0048739	cardiac muscle fiber development	55	2	0.1	4.9e-03
	GO:0070262	peptidyl-serine dephosphorylation	56	2	0.11	5.1e-03
	GO:0060982	coronary artery morphogenesis	61	2	0.12	6.0e-03
	GO:0001822	kidney development	1523	7	2.88	8.5e-03
	GO:0055085	transmembrane transport	3668	9	6.94	1.1e-02
	GO:0042325	regulation of phosphorylation	4917	15	9.3	1.3e-02
3	GO:0032364	oxygen homeostasis	22	2	0.08	3.0e-03
	GO:0010508	positive regulation of autophagy	373	6	1.37	4.1e-03
	GO:0001755	neural crest cell migration	300	5	1.11	5.2e-03
	GO:0072311	glomerular epithelial cell differentiati...	119	2	0.44	7.3e-03
	GO:0002504	antigen processing and presentation of p...	192	2	0.71	7.3e-03
	GO:0036152	phosphatidylethanolamine acyl-chain remo...	39	2	0.14	9.1e-03
	GO:2000001	regulation of DNA damage checkpoint	42	2	0.15	1.1e-02
	GO:0010591	regulation of lamellipodium assembly	163	4	0.6	1.3e-02
	GO:0007049	cell cycle	5368	20	19.78	1.5e-02
	GO:0036119	response to platelet-derived growth fact...	136	2	0.5	1.5e-02
4	GO:0061314	Notch signaling involved in heart develo...	48	3	0.16	5.4e-04
	GO:1990410	adrenomedullin receptor signaling pathwa...	13	2	0.04	8.2e-04
	GO:0002265	astrocyte activation involved in immune ...	15	2	0.05	1.1e-03
	GO:0072554	blood vessel lumenization	16	2	0.05	1.3e-03

	GO:0006811	ion transport	4486	17	14.81	1.5e-03
	GO:0071224	cellular response to peptidoglycan	18	2	0.06	1.6e-03
	GO:1905167	positive regulation of lysosomal protein...	20	2	0.07	2.0e-03
	GO:1902498	regulation of protein autoubiquitination	20	2	0.07	2.0e-03
	GO:1902746	regulation of lens fiber cell differenti...	22	2	0.07	2.4e-03
	GO:0031102	neuron projection regeneration	378	5	1.25	2.5e-03
5	GO:0060178	regulation of exocyst localization	16	3	0.06	3.0e-05
	GO:0098914	membrane repolarization during atrial ca...	34	3	0.13	3.0e-04
	GO:0060672	epithelial cell morphogenesis involved i...	11	2	0.04	7.9e-04
	GO:0001878	response to yeast	50	3	0.19	9.5e-04
	GO:1990410	adrenomedullin receptor signaling pathwa...	13	2	0.05	1.1e-03
	GO:0032484	Ral protein signal transduction	14	2	0.05	1.3e-03
	GO:0016188	synaptic vesicle maturation	74	4	0.28	1.8e-03
	GO:0035881	amacrine cell differentiation	64	3	0.25	2.0e-03
	GO:0031102	neuron projection regeneration	378	4	1.45	3.5e-03
	GO:0019732	antifungal humoral response	23	2	0.09	3.5e-03
6	GO:0050976	detection of mechanical stimulus involve...	30	2	0.04	7.5e-04
	GO:1903818	positive regulation of voltage-gated pot...	49	2	0.07	2.0e-03
	GO:0023041	neuronal signal transduction	59	2	0.08	2.9e-03
	GO:0071286	cellular response to magnesium ion	68	2	0.09	3.8e-03
	GO:1990126	retrograde transport, endosome to plasma...	72	2	0.1	4.3e-03
	GO:0010960	magnesium ion homeostasis	75	2	0.1	4.6e-03
	GO:0006937	regulation of muscle contraction	862	5	1.16	6.4e-03

	GO:0042733	embryonic digit morphogenesis	291	3	0.39	7.1e-03
	GO:0050966	detection of mechanical stimulus involve...	100	2	0.13	8.1e-03
	GO:0090023	positive regulation of neutrophil chemot...	121	2	0.16	1.2e-02
7	GO:0033227	dsRNA transport	33	2	0.03	4.9e-04
	GO:1903715	regulation of aerobic respiration	34	2	0.03	5.3e-04
	GO:0060628	regulation of ER to Golgi vesicle-mediat...	47	2	0.05	1.0e-03
	GO:0035264	multicellular organism growth	835	5	0.83	1.1e-03
	GO:0000212	meiotic spindle organization	62	2	0.06	1.7e-03
	GO:1900049	regulation of histone exchange	10	1	0.01	9.9e-03
	GO:0043007	maintenance of rDNA	10	1	0.01	9.9e-03
	GO:0032763	regulation of mast cell cytokine product...	11	1	0.01	1.1e-02
	GO:0090158	endoplasmic reticulum membrane organizat...	11	1	0.01	1.1e-02
	GO:0007417	central nervous system development	4835	10	4.8	1.1e-02
8	GO:0060013	righting reflex	43	2	0.01	1.9e-05
	GO:0042297	vocal learning	53	2	0.01	2.8e-05
	GO:0060501	positive regulation of epithelial cell p...	54	2	0.01	2.9e-05
	GO:0071625	vocalization behavior	93	2	0.01	8.7e-05
	GO:0021756	striatum development	105	2	0.02	1.1e-04
	GO:0048745	smooth muscle tissue development	168	2	0.03	2.9e-04
	GO:0002053	positive regulation of mesenchymal cell ...	173	2	0.03	3.0e-04
	GO:0033574	response to testosterone	215	2	0.03	4.7e-04
	GO:0048286	lung alveolus development	230	2	0.04	5.3e-04
	GO:0048857	neural nucleus development	238	2	0.04	5.7e-04
9	GO:0031115	negative regulation of microtubule polym...	68	2	0.06	1.6e-03

	GO:0006638	neutral lipid metabolic process	322	2	0.28	5.0e-03
	GO:0051684	maintenance of Golgi location	10	1	0.01	8.6e-03
	GO:0003026	regulation of systemic arterial blood pr...	10	1	0.01	8.6e-03
	GO:0060785	regulation of apoptosis involved in tiss...	11	1	0.01	9.5e-03
	GO:0097176	epoxide metabolic process	11	1	0.01	9.5e-03
	GO:0048877	homeostasis of number of retina cells	11	1	0.01	9.5e-03
	GO:0060738	epithelial-mesenchymal signaling involve...	12	1	0.01	1.0e-02
	GO:0061319	nephrocyte differentiation	12	1	0.01	1.0e-02
	GO:0007228	positive regulation of hh target transcr...	12	1	0.01	1.0e-02
10	GO:0007280	pole cell migration	22	2	0.03	3.5e-04
	GO:0060013	righting reflex	43	2	0.05	1.3e-03
	GO:0042297	vocal learning	53	2	0.07	2.0e-03
	GO:0060501	positive regulation of epithelial cell p...	54	2	0.07	2.1e-03
	GO:0040007	growth	4532	7	5.66	5.6e-03
	GO:0071625	vocalization behavior	93	2	0.12	6.1e-03
	GO:0021756	striatum development	105	2	0.13	7.7e-03
	GO:0048745	smooth muscle tissue development	168	3	0.21	9.9e-03
	GO:0045880	positive regulation of smoothened signal...	125	2	0.16	1.1e-02
	GO:0036303	lymph vessel morphogenesis	108	2	0.13	1.2e-02

Table S3. The first 10 GO terms associated with the differentially expressed mRNA at 200τs and for each contrast.

Contrast	GO ID	Term	Annotated genes	Significant genes	Expected	p-value
SBxSB/PLxPL						
	GO:0031145	anaphase-promoting complex-dependent cat...	158	72	33.72	8.7e-12
	GO:0061418	regulation of transcription from RNA pol...	173	73	36.92	1.1e-10
	GO:0008063	Toll signaling pathway	70	33	14.94	4.0e-10
	GO:0060071	Wnt signaling pathway. planar cell polar...	318	112	67.87	1.0e-09
	GO:0045454	cell redox homeostasis	113	51	24.12	1.3e-08
	GO:0006521	regulation of cellular amino acid metabo...	139	53	29.66	1.4e-08
	GO:0002479	antigen processing and presentation of e...	134	57	28.6	2.7e-08
	GO:0043488	regulation of mRNA stability	431	133	91.98	3.0e-08
	GO:0031146	SCF-dependent proteasomal ubiquitin-depe...	141	59	30.09	3.1e-08
	GO:0071688	striated muscle myosin thick filament as...	65	29	13.87	6.4e-08
PLxSB/PLxPL						
	GO:0046039	GTP metabolic process	145	8	1.36	1.3e-04
	GO:0033955	mitochondrial DNA inheritance	29	4	0.27	1.5e-04
	GO:0006099	tricarboxylic acid cycle	66	5	0.62	3.9e-04
	GO:0018279	protein N-linked glycosylation via aspar...	71	5	0.66	5.5e-04
	GO:0002729	positive regulation of natural killer ce...	41	4	0.38	5.8e-04
	GO:0018401	peptidyl-proline hydroxylation to 4-hydr...	18	3	0.17	6.0e-04
	GO:0002925	positive regulation of humoral immune re...	43	4	0.4	7.0e-04
	GO:0032831	positive regulation of CD4-positive. CD2...	44	4	0.41	7.6e-04
	GO:0051344	negative regulation of cyclic-nucleotide...	21	3	0.2	9.5e-04

	GO:0061762	CAMKK-AMPK signaling cascade	21	3	0.2	9.5e-04
SBXPL/PLxPL						
	GO:0002729	positive regulation of natural killer ce...	41	4	0.19	4.3e-05
	GO:0002925	positive regulation of humoral immune re...	43	4	0.2	5.2e-05
	GO:0032831	positive regulation of CD4-positive. CD2...	44	4	0.21	5.7e-05
	GO:0045954	positive regulation of natural killer ce...	88	5	0.42	6.4e-05
	GO:0046039	GTP metabolic process	145	6	0.69	7.0e-05
	GO:0045588	positive regulation of gamma-delta T cel...	57	4	0.27	1.6e-04
	GO:1901700	response to oxygen-containing compound	6662	33	31.6	1.9e-04
	GO:0033955	mitochondrial DNA inheritance	29	3	0.14	3.5e-04
	GO:0046902	regulation of mitochondrial membrane per...	254	5	1.2	6.3e-04
	GO:0010524	positive regulation of calcium ion trans...	205	5	0.97	8.6e-04
PLxSB/SBxSB						
	GO:0030240	skeletal muscle thin filament assembly	46	20	3.08	3.2e-12
	GO:0045214	sarcomere organization	277	53	18.56	1.6e-10
	GO:0007519	skeletal muscle tissue development	889	123	59.58	2.2e-10
	GO:0071688	striated muscle myosin thick filament as...	65	23	4.36	4.2e-09
	GO:0006096	glycolytic process	262	40	17.56	1.7e-08
	GO:0048741	skeletal muscle fiber development	198	36	13.27	3.0e-08
	GO:0055003	cardiac myofibril assembly	96	25	6.43	4.1e-08
	GO:0043501	skeletal muscle adaptation	128	27	8.58	5.3e-08
	GO:0090131	mesenchyme migration	33	13	2.21	8.5e-08
	GO:0006099	tricarboxylic acid cycle	66	18	4.42	2.1e-07

SBxPL/SBxSB						
	GO:0060827	regulation of canonical Wnt signaling pa...	11	2	0.02	2.7e-04
	GO:0035269	protein O-linked mannosylation	21	2	0.05	1.0e-03
	GO:0031346	positive regulation of cell projection of	1996	5	4.48	6.4e-03
	GO:0009799	specification of symmetry	712	4	1.6	8.8e-03
	GO:0031929	TOR signaling	322	3	0.72	8.8e-03
	GO:0007077	mitotic nuclear envelope disassembly	67	2	0.15	1.0e-02
	GO:0031399	regulation of protein modification proce...	5768	20	12.94	1.2e-02
	GO:0031065	positive regulation of histone deacetyla...	86	2	0.19	1.6e-02
	GO:0010172	embryonic body morphogenesis	96	2	0.22	2.0e-02
	GO:1902477	regulation of defense response to bacter...	10	1	0.02	2.2e-02
PLxSB/SBxPL						
	GO:0006458	'de novo' protein folding	101	15	3.88	3.1e-08
	GO:0000398	mRNA splicing. via spliceosome	694	73	26.65	1.0e-07
	GO:1904874	positive regulation of telomerase RNA lo...	24	9	0.92	1.4e-07
	GO:0006099	tricarboxylic acid cycle	66	14	2.53	1.7e-07
	GO:0061077	chaperone-mediated protein folding	152	21	5.84	1.8e-07
	GO:0006418	tRNA aminoacylation for protein translat...	67	14	2.57	7.4e-07
	GO:0010501	RNA secondary structure unwinding	78	14	2.99	1.4e-06
	GO:0000463	maturation of LSU-rRNA from tricistronic...	43	11	1.65	1.6e-06
	GO:0045727	positive regulation of translation	397	42	15.24	1.8e-06
	GO:0042795	snRNA transcription by RNA polymerase II	117	17	4.49	2.4e-06

Table S4. Putative locations of miRNAs in the clusters of coexpression variability with corresponding *in situ* hybridization data from Wienholds and colleagues, 2005.

Cluster	miRNA family	Location
1	miR-30	Pronephros; cells in epidermis and epithelia of branchial arches; neurons in hindbrain
	miR-203	Most outer layer of epidermis
	miR-200	Nose epithelium; neuromasts; epidermis; pronephric duct; taste buds
	miR-19	Ubiquitous (head, spinal cord, gut, outline somites, neuromasts)
	pmi-miR-9	Proliferating cells of brain, spinal cord and eyes
	miR-181	Brain (tectum, telencephalon); eyes; thymic primordium; gills
	miR-218	Brain (neurons and/or cranial nerves/ganglia in hindbrain); spinal cord
	miR-16	Brain
	miR-204	Neural crest; pigment cells of skin and eye; swimbladder
	miR-27	Cells in branchial arches
2	miR-125	Brain; spinal cord; cranial ganglia
	miR-153	Brain (fore- mid- and hindbrain, diencephalon/hypothalamus)
	miR-16	Brain (tectum); spinal cord; proliferative cells of eyes; pancreas; body muscle
	miR-30	Pronephros; cells in epidermis and epithelia of branchial arches; neurons in hindbrain
	miR-199	Epithelia surrounding cartilage of pharyngeal arches, head skeleton and pectoral fins; epidermis of head; tip of tail
	miR-26	Ubiquitous (head, spinal cord, gut, outline somites, neuromasts)
3	miR-181	Brain (tectum, telencephalon); eyes; thymic primordium; gills
	miR-99	Brain (hindbrain, diencephalon); spinal cord
	miR-126	Bloodvessels and heart
	miR-203	Most outer layer of epidermis
	miR-9	Proliferating cells of brain, spinal cord and eyes
	miR-22	Ubiquitous
	miR-128	Brain (specific neurons in fore- mid- and hindbrain); spinal cord; cranial nerves/ganglia
	miR-199	Epithelia surrounding cartilage of pharyngeal arches, head skeleton and pectoral fins; epidermis of head; tip of tail

	miR-92	Ubiquitous (head, spinal cord, gut, outline somites, neuromasts)
	miR-21	Cardiac valves; otoliths in ears; rhombomere in early stages
	miR-192	Gut and gall bladder, undefined structures in branchial arches
	miR-143	Gut and gall bladder; swimbladder; heart; nose
4	miR-133	Body, head and fin muscles
	miR-375	Brain (pituitary gland); pancreatic islet
	miR-15	Ubiquitous (head, spinal cord, gut, outline somites, neuromasts)
	miR-181	Brain (tectum, telencephalon); eyes; thymic primordium; gills
	miR-20	Ubiquitous (head, spinal cord, gut, outline somites, neuromasts)
	miR-221	Brain (neurons and/or cranial ganglia in forebrain and midbrain); rhombomere in early stages
	miR-222	Brain (neurons and/or cranial ganglia in forebrain and midbrain); rhombomere in early stages
	miR-140	Cartilage of pharyngeal arches,head skeleton and fi
	miR-196	Posterior trunk; later more restricted to spinal cord
	miR-30	Pronephros; cells in epidermis and epithelia of branchial arches; neurons in hindbrain
	miR-181	Brain (tectum, telencephalon); eyes; thymic primordium; gills
	miR-10	Posterior trunk; later more restricted to spinal cord
5	miR-183	Nose epithelium; haircells of neuromasts and ear; cranial ganglia; rods, cones and biar cells of eye; epiphysis
	miR-200	Nose epithelium; neuromasts; epidermis; pronephric duct; taste buds
	miR-199	Epithelia surrounding cartilage of pharyngeal arches, head skeleton and pectoral fins; epidermis of head; tip of tail
	miR-222	Brain (neurons and/or cranial ganglia in forebrain and midbrain); rhombomere in early stages
	miR-196	Posterior trunk; later more restricted to spinal cord
	let-7	Ubiquitous (head, spinal cord, gut, outline somites, neuromasts)
	miR-17	Ubiquitous (head, spinal cord, gut, outline somites, neuromasts)
	miR-140	Cartilage of pharyngeal arches,head skeleton and fins
	miR-203	Most outer layer of epidermis
	miR-181	Brain (tectum, telencephalon); eyes; thymic primordium; gills
	miR-25	Ubiquitous (head, spinal cord, gut, outline somites, neuromasts)

6	miR-10	Posterior trunk; later more restricted to spinal cord
	let-7	Brain; spinal cord
	miR-206	Body, head and fin muscles
	miR-182	Nose epithelium; haircells of neuromasts and ear; cranial ganglia; rods, cones and biar cells of eye; epiphysis
	miR-200	Nose epithelium; neuromasts; epidermis; pronephric duct; taste buds
	miR-203	Nose epithelium; neuromasts; epidermis; pronephric duct; taste buds
	miR-205	Epidermis; epithelia ofpharyngeal arches; intersegmental cells; not in sensory epithelia
7	miR-30	Pronephros; cells in epidermis and epithelia of branchial arches; neurons in hindbrain
	miR-203	Most outer layer of epidermis
	miR-200	Nose epithelium; neuromasts; epidermis; pronephric duct; taste bud
	miR-19	Ubiquitous (head, spinal cord, gut, outline somites, neuromasts)
	miR-9	Proliferating cells of brain, spinal cord and eyes
	miR-181	Brain (tectum, telencephalon); eyes; thymic primordium; gills
	miR-16	Brain
	miR-204	Neural crest; pigment cells of skin and eye; swimbladder
	miR-27	Cells in branchial arches
8	miR-184	Lens; hatching gland; epidermis
9	miR-100	Brain (hindbrain, diencephalon); spinal cord
	miR-92	Ubiquitous (head, spinal cord, gut, outline somites, neuromasts)
10	miR-100	Brain (hindbrain, diencephalon); spinal cord
	miR-21	Cardiac valves; otoliths in ears; rhombomere in early stages
	miR-184	Lens; hatching gland; epidermis

Table S5. The first 10 GO terms of genes with maternal patern of expression and of gene with expression biased towards the PL-morph in the hybrids, at 200τs.

	GO ID	Term	Annotated genes	Significant genes	Expected	P
Maternal	GO:0015917	aminophospholipid transport	14	2	0.03	3.0e-04
	GO:0035999	tetrahydrofolate interconversion	19	2	0.04	5.7e-04
	GO:0070268	cornification	98	3	0.18	8.2e-04
	GO:0070863	positive regulation of protein exit from...	23	2	0.04	8.4e-04
	GO:0097264	self proteolysis	55	2	0.1	4.7e-03
	GO:0045214	sarcomere organization	277	3	0.51	1.5e-02
	GO:0051892	negative regulation of cardioblast diffe...	19	2	0.04	1.6e-02
	GO:0046716	muscle cell cellular homeostasis	105	2	0.2	1.6e-02
	GO:0007517	muscle organ development	1944	7	3.61	1.8e-02
	GO:0003365	establishment of cell polarity involved ...	10	1	0.02	1.8e-02
PL-biased	GO:0009799	specification of symmetry	712	2	0.8	4.5e-03
	GO:0010506	regulation of autophagy	951	5	1.07	9.0e-03
	GO:1905209	positive regulation of cardiocyte differ...	141	2	0.16	1.1e-02
	GO:1902477	regulation of defense response to bacter...	10	1	0.01	1.1e-02
	GO:0036306	embryonic heart tube elongation	10	1	0.01	1.1e-02
	GO:0009272	fungal-type cell wall biogenesis	10	1	0.01	1.1e-02
	GO:0060827	regulation of canonical Wnt signaling pa...	11	1	0.01	1.2e-02
	GO:0060577	pulmonary vein morphogenesis	11	1	0.01	1.2e-02
	GO:1990569	UDP-N-acetylglucosamine transmembrane tr...	11	1	0.01	1.2e-02
	GO:0060468	prevention of polyspermy	11	1	0.01	1.2e-02

Table S6. Name and location of the genes showing a biased expression in hybrids towards the PL-morph, at 150τs and 200τs.

	Symbol	Name	Chromosome
150τs			
	LOC111950271	immunoglobulin superfamily DCC subclass member 3-like	LG23
	tp53	tumor protein p53	LG23
	LOC111951066	tumor necrosis factor receptor superfamily member 19	LG23
	LOC111952141	40S ribosomal protein S27-like	LG26
	LOC111956165	immunoglobulin superfamily DCC subclass member 3-like	LG31
	LOC111976118	40S ribosomal protein S27-like	LG16
	adprh	ADP-ribosylarginine hydrolase	LG2
	LOC111980339	tumor necrosis factor receptor superfamily member 19-like	LG20
	LOC111981653	uncharacterized LOC111981653	LG20
	LOC112069817	cyclin-G1-like	Un
	LOC112072678	uncharacterized LOC112072678	Un
	sybl1	synaptobrevin-like 1	Un
200τs			
	kera	keratocan	LG24
	LOC111955992	splicing factor U2AF 65 kDa subunit	LG31
	LOC111956824	UDP-N-acetylglucosamine transporter	LG32
	bmp16	bone morphogenetic protein 16	LG4p
	LOC111960782	low choriolytic enzyme-like	LG4p
	LOC111962715	nuclear pore complex protein Nup160-like	LG4q.1:29
	LOC111963418	protein Daple-like	LG4q.2
	LOC111965102	semaphorin-4D-like	LG6.1
	xkrx	XK related X-linked	LG6.2
	zgc:110843	CDGSH iron-sulfur domain-containing protein	LG6.2
	LOC111967380	myozenin-2-like	LG8
	LOC111967383	HLA class II histocompatibility antigen gamma chain	LG8
	LOC111967891	MKL/myocardin-like protein 1	LG8

LOC111968855	filamin-A-interacting protein 1	LG9
LOC111969506	switch-associated protein 70	LG10
LOC111969927	store-operated calcium entry regulator STIMATE	LG11
fkbp5	FKBP prolyl isomerase 5	LG11
LOC111972571	vesicular, overexpressed in cancer, prosurvival protein 1	LG14
mtch2	mitochondrial carrier homolog 2	LG15
adprh	ADP-ribosylarginine hydrolase	LG2
LOC111978879	ras-related protein Rab-8A-like	LG19
LOC111979494	Niemann-Pick C1 protein	LG19
LOC111981097	parvalbumin-7	LG20
LOC111982785	claudin-4-like	LG22
vgll2b	vestigial-like family member 2b	Un
LOC112069671	trans-Golgi network integral membrane protein 1-like	Un
LOC112069734	calponin-3-like	Un
mybpc2a	myosin binding protein Ca	Un
LOC112072130	transcription factor EB-like	Un
znf106a	zinc finger protein 106a	Un
LOC112075995	ncRNA	Un
LOC112076473	oocyte zinc finger protein XlCOF6-like	Un
LOC112077739	ncRNA	Un
LOC112078614	uncharacterized LOC112078614	Un
SDF2L1	stromal cell derived factor 2 like 1	22
TBX15	T-box transcription factor 15	1
SLC10A7	solute carrier family 10 member 7	4
MLIP	muscular LMNA interacting protein	6

Table S7. The first 10 GO terms of the first 5 putative targets of each differentially expressed miRNAs.

Contrast	GO ID	GO Term	Annotated genes	Significant genes	Expected	p-value
SBxSB/PLXPL 150τ_s	GO:1902746	regulation of lens fiber cell differenti...	22	2	0.04	5.7e-04
	GO:1901844	regulation of cell communicatio n by elec...	33	2	0.05	1.3e-03
	GO:0060017	parathyroid gland development	38	2	0.06	1.7e-03
	GO:0014911	positive regulation of smooth muscle cel...	215	4	0.34	2.3e-03
	GO:0032754	positive regulation of interleukin-5 pro...	45	2	0.07	2.4e-03
	GO:0045602	negative regulation of endothelial cell ...	56	2	0.09	3.7e-03
	GO:0048103	somatic stem cell division	68	2	0.11	5.4e-03
	GO:0071732	cellular response to nitric oxide	78	2	0.12	7.0e-03
	GO:0042325	regulation of phosphorylation	4917	14	7.88	8.6e-03
	GO:0001771	immunological synapse formation	87	2	0.14	8.6e-03
PLxSB/PLxPL 150τ_s[1]	NA	NA	NA	NA	NA	NA
SBxPL/PLxPL 150τ_s	GO:0010992	ubiquitin recycling	12	1	0	1.5e-03
	GO:0060340	positive regulation of type I interferon...	13	1	0	1.7e-03
	GO:0051661	maintenance of centrosome location	17	1	0	2.2e-03
	GO:0044791	positive regulation by host of viral rel...	22	1	0	2.8e-03
	GO:0033182	regulation of histone ubiquitination	36	1	0	4.6e-03
	GO:0000729	DNA double-strand break processing	40	1	0.01	5.1e-03

	GO:0035458	cellular response to interferon-beta	58	1	0.01	7.4e-03
	GO:0051443	positive regulation of ubiquitin-protein...	95	1	0.01	1.2e-02
	GO:0010800	positive regulation of peptidyl-threonin...	95	1	0.01	1.2e-02
	GO:0006301	postreplication repair	114	1	0.01	1.5e-02
PLxSB/SBxSB	GO:0003026	regulation of systemic arterial blood pr...	10	2	0.03	4.4e-04
150τ_s	GO:0000301	retrograde transport. vesicle recycling ...	11	2	0.03	5.4e-04
	GO:0097401	synaptic vesicle lumen acidification	11	2	0.03	5.4e-04
	GO:1902010	negative regulation of translation in re...	12	2	0.04	6.4e-04
	GO:1904504	positive regulation of lipophagy	17	2	0.05	1.3e-03
	GO:0050915	sensory perception of sour taste	18	2	0.06	1.5e-03
	GO:0071233	cellular response to leucine	19	2	0.06	1.7e-03
	GO:1990253	cellular response to leucine starvation	20	2	0.06	1.8e-03
	GO:0042325	regulation of phosphorylation	4917	23	15.6	1.9e-03
	GO:0036091	positive regulation of transcription fro...	22	2	0.07	2.2e-03
SBPL/SBxSB	GO:1902746	regulation of lens fiber cell differenti...	22	3	0.05	1.5e-05
150τ_s	GO:0010992	ubiquitin recycling	12	2	0.03	3.0e-04
	GO:0090084	negative regulation of inclusion body as...	24	2	0.05	1.3e-03
	GO:0035333	Notch receptor processing. ligand-depend...	37	2	0.08	3.0e-03
	GO:0042026	protein refolding	37	2	0.08	3.0e-03
	GO:0071514	genetic imprinting	84	3	0.18	3.0e-03

	GO:0006977	DNA damage response. signal transduction...	147	3	0.32	4.1e-03
	GO:0045602	negative regulation of endothelial cell ...	56	2	0.12	6.7e-03
	GO:0007096	regulation of exit from mitosis	60	2	0.13	7.6e-03
	GO:0045736	negative regulation of cyclin-dependent ...	61	2	0.13	7.9e-03
PLxPL/SBxSB 200τ_s	GO:0061386	closure of optic fissure	32	4	0.27	1.5e-04
	GO:0033128	negative regulation of histone phosphory...	18	3	0.15	4.4e-04
	GO:2001045	negative regulation of integrin-mediated...	20	3	0.17	6.1e-04
	GO:0042311	vasodilation	147	6	1.24	1.4e-03
	GO:0009630	gravitropism	12	2	0.1	4.5e-03
	GO:0050965	detection of temperature stimulus involv...	82	4	0.69	5.2e-03
	GO:0048484	enteric nervous system development	85	4	0.72	5.9e-03
	GO:0071475	cellular hyperosmotic salinity response	16	2	0.14	7.9e-03
	GO:0035279	mRNA cleavage involved in gene silencing...	16	2	0.14	7.9e-03
	GO:0046101	hypoxanthine biosynthetic process	16	2	0.14	7.9e-03
PLxSB/PLxPL 200τ_s	GO:0070863	positive regulation of protein exit from...	23	2	0.06	1.4e-03
	GO:0010587	miRNA catabolic process	28	2	0.07	2.1e-03
	GO:0031054	pre-miRNA processing	30	2	0.07	2.4e-03
	GO:0018298	protein-chromophore linkage	33	2	0.08	2.9e-03
	GO:0042487	regulation of odontogenesis of dentin-co...	39	2	0.09	4.1e-03
	GO:2000001	regulation of DNA damage checkpoint	42	2	0.1	4.7e-03

	GO:0045664	regulation of neuron differentiation	3440	12	8.38	5.6e-03
	GO:0014911	positive regulation of smooth muscle cel...	215	4	0.52	7.5e-03
	GO:0060080	inhibitory postsynaptic potential	107	3	0.26	7.5e-03
	GO:0031098	stress-activated protein kinase signalin...	1259	5	3.07	7.7e-03
SBxPL/PLxPL **200τ_s**	GO:0035338	long-chain fatty-acyl-CoA biosynthetic p...	52	2	0.06	1.9e-03
	GO:0030206	chondroitin sulfate biosynthetic process	69	2	0.09	3.4e-03
	GO:0036303	lymph vessel morphogenesis	108	2	0.13	1.2e-02
	GO:1905203	regulation of connective tissue replacem...	10	1	0.01	1.2e-02
	GO:0001869	negative regulation of complement activa...	10	1	0.01	1.2e-02
	GO:0033037	polysaccharide localization	11	1	0.01	1.4e-02
	GO:1901995	positive regulation of meiotic cell cycl...	11	1	0.01	1.4e-02
	GO:0009630	gravitropism	12	1	0.01	1.5e-02
	GO:0072530	purine-containing compound transmembrane...	12	1	0.01	1.5e-02
	GO:0030263	apoptotic chromosome condensation	13	1	0.02	1.6e-02
PLxSB/SBxSB **200τ_s**	GO:0048484	enteric nervous system development	85	2	0.09	3.9e-03
	GO:0043321	regulation of natural killer cell degran...	10	1	0.01	1.1e-02
	GO:1900063	regulation of peroxisome organization	10	1	0.01	1.1e-02
	GO:2000276	negative regulation of oxidative phospho...	10	1	0.01	1.1e-02
	GO:0036180	filamentous growth of a population of un...	10	1	0.01	1.1e-02

	GO:1901995	positive regulation of meiotic cell cycl...	11	1	0.01	1.2e-02
	GO:0035359	negative regulation of peroxisome prolif...	11	1	0.01	1.2e-02
	GO:0010992	ubiquitin recycling	12	1	0.01	1.3e-02
	GO:0061945	regulation of protein K48-linked ubiquit...	12	1	0.01	1.3e-02
	GO:0009785	blue light signaling pathway	12	1	0.01	1.3e-02
SBxPL/SBxSB						
200τ_s[1]	NA	NA	NA	NA	NA	NA

[1]No target corresponding to expressed genes in the mRNA dataset.

Table S8. Scores and percentages of alignment of the three miRNAs matching 3' UTR target sites of *bmp4**.

miRNA	Total Score	Total Energy	Alingnement 1	Alignement 2
efu-miR-101	182	-31.29	73.91%	91.30%
ssa-miR-21a-2-3p	172	-26.78	84.21%	94.74%
ccr-miR-459-5p	174	-19.05	84.21%	89.47%

* Sequence: NC_036862.1:6137142-6138894 for the ASM291031v2 genome assembly

Table S9. Fold change and *P*-values of *ptch1* and *bmp4* for each cross vs. cross contrast.

Gene	Contrast	Base mean	$\log_2$ fold change	*P*	adjusted *P*
bmp4	PLxPL vs. SBxSB	64.14	-0.43	0.00	0.00
	PLxSB vs. PLxPL	64.14	-0.08	0.11	0.43
	SBxPL vs. PLxPL	64.14	-0.06	0.04	0.46
	PLxSB vs. SBxSB	64.14	0.19	0.01	0.08
	SBxPL vs. SBxSB	64.14	0.11	0.02	0.29
ptch1	PLxPL vs. SBxSB	201.67	-0.25	0.01	0.04
	PLxSB vs. PLxPL	201.67	-0.10	0.03	0.28
	SBxPL vs. PLxPL	201.67	-0.02	0.42	0.85
	PLxSB vs. SBxSB	201.67	0.03	0.55	0.82
	SBxPL vs. SBxSB	201.67	0.09	0.05	0.39

Table S10. Rationale to indentify the putative dominance of candidate genes according to fold changes and adjusted P-values.

Dominance	Contrast	Log_2 fold change*	Adjusted P
Maternal	SBxSB vs. PLxPL	<0	<0.1
	SBxPL vs. PLxPL	<0	<0.1
	PLxSB vs. PLxPL	-	>0.1
	SBxPL vs. SBxSB	-	>0.1
	PLxSB vs. SBxSB	>0	<0.1
PL-dominant	SBxSB vs. PLxPL	<0	<0.1
	SBxPL vs. PLxPL	-	>0.1
	PLxSB vs. PLxPL	-	>0.1
	SBxPL vs. SBxSB	>0	<0.1
	PLxSB vs. SBxSB	>0	<0.1
SB-dominant	SBxSB vs. PLxPL	<0	<0.1
	SBxPL vs. PLxPL	>0	<0.1
	PLxSB vs. PLxPL	>0	<0.1
	SBxPL vs. SBxSB	-	>0.1
	PLxSB vs. SBxSB	-	>0.1
Additive	SBxSB vs. PLxPL	<0	<0.1
	SBxPL vs. PLxPL	<0	<0.1
	PLxSB vs. PLxPL	<0	<0.1
	SBxPL vs. SBxSB	>0	<0.1
	PLxSB vs. SBxSB	>0	<0.1
Overdominant	SBxSB vs. PLxPL	-	-
	SBxPL vs. PLxPL	>0	<0.1
	PLxSB vs. PLxPL	>0	<0.1
	SBxPL vs. SBxSB	>0	<0.1
	PLxSB vs. SBxSB	>0	<0.1

Underdominant	SBxSB vs. PLxPL	-	-
	SBxPL vs. PLxPL	<0	<0.1
	PLxSB vs. PLxPL	<0	<0.1
	SBxPL vs. SBxSB	<0	<0.1
	PLxSB vs. SBxSB	<0	<0.1

* $\log_2$ fold change > 0 corresponds to overexpression in the cross type on left. For simplicity, only $\log_2$ fold change < 0 in SBxSB vs. PLxPL are shown.

Paper IV

Asymmetric reproductive isolation in sympatric Arctic charr morphs revealed by a comprehensive examination of reproductive barriers.

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Abstract.

Polymorphic fishes from postglacial lakes are ideal models for speciation studies, but little is known on the evolution of reproductive barriers in these systems. We characterised reproductive isolation and the absolute and relative strengths of reproductive barriers between two sympatric populations with a recent evolutionary history but extensive phenotypic and genetic differences: the planktivorous and the small-benthic morph of Arctic charr (*Salvelinus alpinus*) from Thingvallavatn, Iceland. We estimated the importance of reproductive barriers through a fishing survey, a mate choice experiment and a common-garden experiments involving the offspring of each morph and F_1 hybrids. We observed compelling evidence for asymmetric gene flow between the two morphs. Reproductive isolation was virtually complete in the planktivorous charr because of their use of the spawning habitats over time. However, this barrier was ineffective in the small-benthic charr, for which assortative mating and postmating isolation (fertilization failures and/or embryo mortality) tended to induce partial reproductive isolation. While these results support to some extent the view that premating barriers appear first during divergence, our study also showed that intrinsic postzygotic barriers are likely to evolve early during the processes of ecological speciation.

Introduction.

Over the past decade, much progress has been made in understanding the gradual accumulation and the strengthening reproductive barriers between diverging populations, that are, the evolution of reproductive isolation along the speciation continuum (Hendry, 2009; Nosil, 2012b; Seehausen et al., 2014; Stankowski & Ravinet, 2021). However, the general mechanisms driving the build-up of reproductive isolation remain difficult to comprehend. This difficulty especially resides in that the increase reproductive of isolation with genetic divergence is not linear, is poorly explained by phenotypic differences or ecological setups, do not operate at the same rate among taxa, and varies depending on the type of barriers involved (Matute & Cooper, 2021; Rabosky & Matute, 2013; Stankowski & Ravinet, 2021). Studies fully characterising reproductive isolation among populations at a

given point along the speciation continuum are clearly required, and need to extend beyond the main biological models, *i.e.*, *Drosophila* (Butlin et al., 2012; Matute & Cooper, 2021). Diverging populations or species with recent and simple evolutionary histories are especially well-suited for unravelling the evolution of reproductive isolation under divergent selection while avoiding the confounding effects of drift (Foote, 2017; Seehausen & Wagner, 2014).

Freshwater fishes are acclaimed models of adaptation to a multitude of environments, which typically involve trophic differences among populations, the latter often segregating between benthic and pelagic habitats (Seehausen & Wagner, 2014). Such divergence patterns are especially observed across the Northern hemisphere, where populations occupying deglaciated lakes exhibit a wide range of phenotypic variations and levels of reproductive isolation (Doenz et al., 2018; Hendry, 2009; Lackey & Boughman, 2017; Ólafsdóttir et al., 2006; Schluter, 1996; Skúlason et al., 2019; Smith & Skúlason, 1996). Illustrative cases of polymorphism have notably been discovered in Salmonids, which have recently been the focus of extensive morphological and in genomics studies (Doenz et al., 2018; Evgeny et al., 2018; Østbye et al., 2020; Salisbury & Ruzzante, 2022). However, thorough studies on the nature and the relative importance of reproductive barriers within these systems are missing.

Here we estimated reproductive isolation and the relative importance of different reproductive barriers in Arctic charr morphs (*Salvelinus alpinus*) of Thingvallavatn in Iceland. Two of the four Arctic charr morphs coexisting in Thingvallavatn, the planktivorous (PL) and the small-benthic charr (SB), constitute a prime system for speciation. Despite a short evolutionary time frame these morphs differ extensively in a suit of phenotypic traits relating to resource use. The morphs differ in head and body shape (Kapralova et al., 2015; Parsons et al., 2010; Skúlason, Noakes, et al., 1989; Snorrason et al., 1994), diet (Malmquist et al., 1992), habitat use (Sandlund et al., 1987), feeding behaviour (Malmquist, 1992; Skúlason et al., 1993), growth pattern, life-history (Jonsson et al., 1988; Sandlund et al., 1992; Snorrason et al., 1994) and parasites (Franklin, 2017). Genetic studies indicate strong reproductive isolation of PL- and SB-charr (Guðbrandsson et al., 2019; Kapralova et al., 2011; Snorrason et al., 1994), yet both morphs are found to spawn in the stony littoral zone and their time of spawning overlap to a large degree (Skúlason, Noakes, et al., 1989). Signs of strong genetic differentiation are spread throughout the two charr transcriptomes (Guðbrandsson et al., 2019) and genomic data indicates that 9.5% of hybrids may occur in the their combined populations (Brachmann et al., 2021). Viable hybrids can be reared in laboratory conditions, but selection against hybrids appears likely according to recent findings of dominance in morphology, growth and personality syndromes, and gene expression (De la Cámara et al., 2021; Horta-Lacueva et al., 2020, 2021).

We identified pre- and postzygotic barriers between PL- and SB-charr by conducting fishing surveys and mating experiments, and through common-garden experiments involving the offspring of each morph and reciprocal hybrids. We used all these data to calculate indices of the absolute strength of these barriers and of their relative contribution to total reproductive isolation.

Material and Methods.

Study system.

Thingvallavatn is an oligotrophic lake sitting in a graben of the Mid-Atlantic ridge in SW Iceland (area = 83km^2; mean depth = 34m, max. depth = 114m). The present lake formed following the last glacial retreat about 10,000 years ago, and has been subjected to changes due to tectonic and volcanic activities since then (Pétursson et al., 2015; Sæmundsson, 1992). The physical structure of the lake is characterized by an extensive pelagic zone and three major benthic habitats; a "stony littoral" zone (0-10m deep), a zone consisting of stands of the green alga *Nitella opaca* (10-20m deep), and a profundal zone (25 m and deeper) covered by a diatomic gyttja substrate (Sandlund et al., 1992). The four Arctic charr morphs of Thingvallavatn belong to two ecotypes (Snorrason et al., 1989): a benthic ecotype, including the small and the large benthic morphs (*i.e.*, SB- and LB-charr), and a pelagic ecotype including the planktivorous and the piscivorous morphs (*i.e.,* PL- and PI-charr). SB-, LB- and PL-charr constitute three genetically differentiated populations, the status of PI-charr remaining unresolved (Jóhannes Guðbrandsson et al., 2019). All four morphs are currently sympatric, although coalescent simulations suggest a short initial period of geographic isolation between PL- and SB-charr (Kapralova et al., 2011). LB-charr spawn earlier than all three other morphs (late July- mid-August) while the spawning season of SB-charr extends over a long period (August-November) and overlaps with the more synchronous spawning time (mid-September – mid-October) of PL-charr (Skúlason, Snorrason, et al., 1989). Age-0 juveniles of both PL- and SB-charr are believed to occupy the littoral zone of the lake until summer, when PL-charr migrate to deeper zones (Sandlund et al., 1988).

We studied the two best known spawning grounds of Thingvallavatn: a stretch of shallow, submerged lava in the north-eastern shore in the lake (Ólafsdráttur) and a small bay on the eastern shore north of the Mjóanes peninsula. The two sites are located about 6km apart from one-another.

Premating barrier: Spatiotemporal isolation.

We conducted a fishing survey over the 2017 breeding season. We laid nets weekly overnight at the two best studied spawning sites (Mjóanes and Ólafsdráttur), from the first week of September to the first week of November. Fishing consisted of multi-meshed nets consisting of 6 panels (6m long and 1.8m deep with mesh sizes 10, 12.5, 15.5, 19, 22 and 25mm, knot to knot). 1137 specimens were obtained and dissected to assess gonadal ripeness. Males with sperm or empty testes reaching the anus were considered ripe. Females were considered ripe if we observed loose eggs in the body cavity.

Premating barrier: Mate choice.

We conducted a choice experiment confronting one SB- or PL-female with two males, one of each morph. The fishes were caught during the 2018 spawning season and transported to the research facilities of Sudurnes Science and Leanring Center (Sandgerði, Iceland). The experimental setup consisted of a 160L (150x40x40cm) glass tanks with continuous water input, containing rocks to mimic the spawning environment, and a rigid, horizontal mesh over the bottom to prevent the fishes from eating the eggs produced during the interactions.

The female was introduced first and was given one hour of habituation, after which the males were added and left to interact freely for about eleven hours. The interactions were continuously recorded with an infrared surveillance camera (Foscam 1080P) positioned above the container. 24 observation trials (involving 12 with SB-females and 12 with PL-females with one female and a male of each morph) could be successfully recorded. Because SB-charr are difficult to collect, we reused 7 of the SB-males up to 3 times. After each trial the eggs produced were counted and the fish were killed with a blow to the head.

Arctic charr exhibit stereotyped courtship behaviours and male-male agonistic interactions for accessing females (Fabricius, 1953; Sigurjónsdóttir & Gunnarsson, 1989), facilitating the use of behavioural event as proxies for mate choice studies. Courtship events were recorded in 18 trials of the 24 trials, and males from both morphs managed to court at least once with the female in 8 trails. Therefore, we attributed the outcome of the mating trial to the male with the highest number of courtship. Courtship events were categorized using the behaviour descriptions from (Sigurjónsdóttir & Gunnarsson, 1989), and were quantified with the BORIS software (Friard & Gamba, 2016).

Postmating barriers.

We estimated the fertility success (a postmating, prezygotic barrier) and early hybrid survival (premating barrier) by tracking the ontogeny of PL-, SB-charr and reciprocal hybrids reared in common garden conditions. We collected adult specimens in October 2017 over the spawning grounds of Mjóanes. We crossed the gametes of 50 ripe specimens as soon as they were brought ashore to make 25 families (Female x Male parents: 5 PLxPL; 5 SBxSB; 9 PLxSB; 6 SBxPL). The fertilised eggs were immediately transported to a hatching tray at the Institute of Experimental Pathology at Keldur, University of Iceland. We monitored the eggs daily, counting and removing dead eggs and those that had not been fertilized. When embryos could be observed directly (*i.e.*, when pigmentation reached the entire surface of the eyes) and thereby, fertilisation could be confirmed, we transported the eggs to a common vertical incubator (MariSource, USA) in Verið, the aquaculture facilities of Hólar University, Sauðárkrókur, Iceland. We monitored the embryos daily for mortality and hatching. Two months after hatching, 1045 embryos from 11 families (3 families in each of the PLxPL, SBxSB and PLxSB cross; mean number of individuals per family = 95; max = 239 ; min = 20) were killed, measured to fork length and used for a companion study internal on craniofacial morphology (Ponsioen, 2020). We placed 214 other embryos from 17 families (4 PLxPL, 7 PLxSB, 3 SBxSB and 3 SBxPL families; mean number of individuals per family = 12.6; max = 20 ; min = 7) into large plastic buckets (35 cm deep x 29 cm diameter) that were continuously supplied with water. At the onset of exogeneous feeding, we fed juveniles daily with aquaculture pellets. At 5- and 7-months post-hatching (i.e., 9 and 10 month post-fertilisation), an average of 12 individuals per family were sampled, measured and return to the water. All individuals were killed and frozen 10 months after hatching. Once defrosted, the specimens were weighted, measured to fork length with a measuring tape, photographed on the left lateral side (Canon 1100D, 90mm 1:2.8 Tamron lens MACRO 1:1) and dissected for wet liver weight. Anaesthesia for life measurements and euthanasia was done with 2-phenoxyethanol according to the recommendations for salmonids (Pounder, Mitchell, Thomson, Pottinger, & Sneddon, 2018)

Analyses of postzygotic isolation.

Besides pre-hatching mortality, we tested the hypothesis of intrinsic hybrid inviability by comparing individual body condition among cross types ten month after hatching. We used weight-length relationship (García-Berthou, 2001), wet liver weight, and body morphology as proxies for body condition. We also tested for characteristics putatively associated with ecological (extrinsic) hybrid inviability by comparing crosses for growth (from two to ten months after hatching) and body morphology (ten months after hatching).

For linear measurements, we fitted Generalized Linear Mixed Models using the R package MCMCglmm (Hadfield, 2010). We specified weakly informative priors ($V_{0family}$ = 1, V_{0ind}, V_{res} = identity matrix, nu = 0.002) and determined the optimal number of iterations by examining trace plots, posterior density plots and effective sample sizes. Model formulae are described in Table S1. These analyses were made within the Bayesian framework, inferences were made by comparing the posterior modes, the 95% credible intervals (CrI) and the posterior densities of the estimated values.

For morphometric data, we digitized the photographed specimens with the R tools Stereomorph (Olsen & Westneat, 2015). We digitized 8 landmarks and extracted semi-landmarks from Bezier curves: 4 around the eye, 10 around the head and 4 along the ventral side (Fig. 1). We analysed shape variations with Procrustes analyses with randomized residual permutation procedure in R, using Geomorph (Adams & Otárola-Castillo, 2013; Adams et al., 2013).

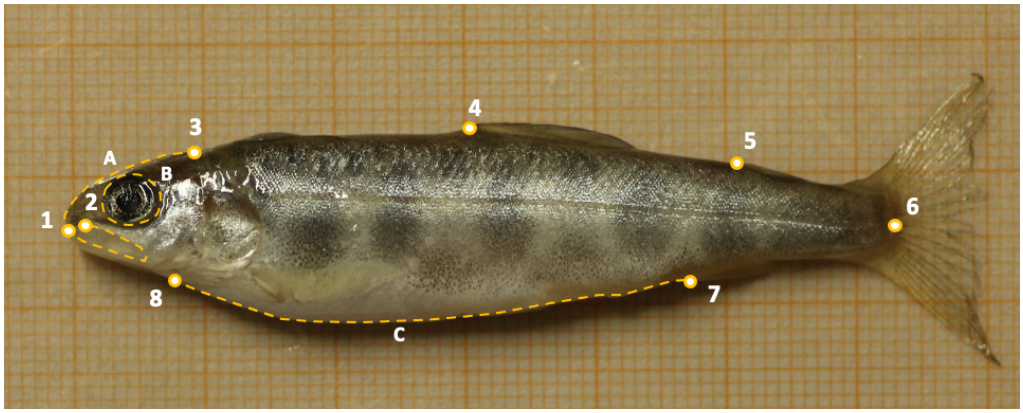


Fig. 1. Landmarks used estimate shape. Landmarks: (1) Tip of the lower jaw, (2) anterior upper edge of the maxilla, (3) intersection of the brain with the supraoccipital, (4) the anterior edge of the dorsal fin and (5) of the adipose fin, (6) extremity of the notochord, (7) the anterior edge of anal fin the intersection, (8) intersection of the ventral side with the operculum. Curves: (A) Maxilla, snout and brain, (B) eye, (C) stomach.

Reproductive isolation estimates.

We estimated absolute and relative reproductive isolation estimates (RI) for the different reproductive barriers (Table 1) according to equation (4) in (Sobel & Chen, 2014). Briefly, this equation describes a linear relationship between RI and the probability of gene flow, using estimates of "heterospecific mating"/surviving hybrids (*H*) and number of "conspecific" mating/surviving conspecific offspring (*C*). RI range from 1 (no gene flow) to -1 (gene flow facilitated by mating behaviours and/or heterosis), with RI = 0 for random mating and no postmating isolation nor hybrid advantage (probability of gene flow = 0.5).

Table 1. Reproductive barriers and proxies to calculate their related RI estimates.

Type of reproductive barrier	**Reproductive barrier**	**Data**	**Proxy**
Prezygotic	Spatiotemporal segregation during spawning	Fishing survey data	Probability of encounter during spawning
Prezygotic	Assortative mating	Mate choice experiment	Courtship intensity
Postzygotic (and post-mating prezygotic)	Hybrid inviability	Common garden experiment	Mortality of embryos and juveniles Body condition

Because the sex ratio and the number of individuals of both morphs varied over time and between spawning sites, we estimated *C* and *H* as the number of eggs fertilized during heterospecific and conspecific encounters, such as:

$$C_a = \sum_{i=1}^{a} \left\{ \left[P\left(Fe_{a,i}\right) \times P(Ma_{a,i}) \right] \times n_{a,i} \times F_a \right\}$$

$$H_a = \sum_{i=1}^{a} \left\{ \left[P\left(Fe_{a,i}\right) \times P(Ma_{b,i}) \right] \times n_{a,i} \times F_a \right\}$$

with *a,b* morphs, *i* the sampling date, *n* the number of mature females, *P* the probabilities of drawing a female (*Fe*) and a male (*Ma*) of a given morph, and *F* the mean individual fecundity for the population. We calculated *F* using morph-specific regressions of *F* on length and age from (Jonsson et al., 1988), using the mean length of each morph and at each site from our survey data, and the mean age of mature individuals estimated from otolith data from a 2019 survey. We assumed that each charr mated once and during the first encounter. We also assume that SB-charr did not spawn outside of the sampling period. We also relied on the assumption that the numbers of specimens caught during the survey accurately reflect the relative abundance or the activity of the fishes, and are not biased by size differences between morphs. Finally, we assumed that the size distributions of the fishes caught during the survey are representative of the wild populations.

We estimated the total RI from each morph using multiplicative products of the *H* and C terms of each barrier in the RI formula (Sobel & Chen, 2014). We then calculated the absolute strength of individual reproductive barriers sequentially (so the strength of a given barrier only contributes to the portion of total RI than is not explained by barriers occurring earlier in the life cycle of the organism; Ramsey, Bradshaw, & Schemske, 2003). Finally, we calculated the relative strength of each barrier as its absolute contribution divided by the total RI (Sobel & Chen, 2014). Because of the uncertainty in the patterns of assortative mating and fertilisation underlined by modest samples sizes (see below), we considered two scenarios when analysing reproductive isolation: a scenario with spatiotemporal isolation and assortative mating only (but from which conservative indices of spatiotemporal isolation alone can be extracted), and a scenario combining spatiotemporal isolation, assortative mating and postzygotic isolation mechanisms.

Results.

Spatiotemporal isolation.

The fishing survey data indicated some differences between the morphs in the use of the two spawning sites. These differences featured both the timing of arrival (rise in density) and the number of individuals of each sex (Fig. 2). First, the two morphs partially differ in spawning time, with a peak in spawning SB-charr numbers in early September, followed by a slow increase in mature PL-charr until early to mid-October. Second, we observed a clear difference between the two sites, most female PL-charr being captured in Mjóanes while SB-charr were mainly caught in Ólafsdráttur.

Mate choice.

The females recorded in the mate choice experiment courted the most with the male of their own morph (Table 1). This pattern was not statistically significant (Fisher's exact test on data excluding trial with no courtship event: $p = 0.15$). Note, however, that two of the three SB-females that mated with PL-males were very large specimens (184, 158 and 136mm; average size of SB-females in the experiment = 134mm).

Mate choice exhibited from males differed between morphs. SB-males courted females of their own morph more than females of the other morph (one-sided Wilcoxon test, $W = 35$; $p = 0.01$; Fig S.1). In PL-males, we did not observe evidence for differences in male courtship event counts depending on the morph of the female (two-sided Wilcoxon test, $W = 77.5$; $p = 0.76$; Fig. S1).

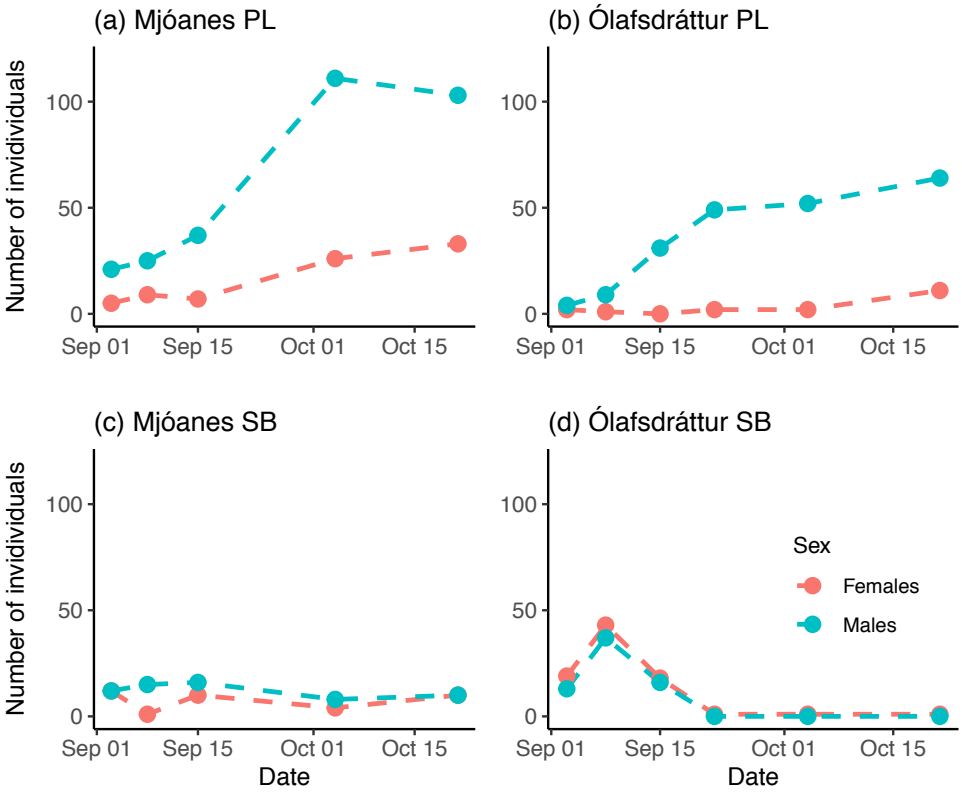


Fig. 2. Number of individuals collected during the fishing survey. PL-charr (a,b) and SB-charr (c,d) at the spawning sites of Mjóanes (a,c) and Ólafsdráttur (b,d).

Table 1. Number of mating trials in which the female courted more with the male of a given morph[1].

Choice	**PL-females**	**SB-females**
PL-male	6 (5)	3
SB-male	2	7
No choice	4	2

[1]Numbers in brackets: Trials with completed mating (expulsed eggs).

Fertilisation and early survival.

Survival and fertilisation success appeared to be reduced in hybrids compared to pure-morph crosses (Fig. 3). This pattern corresponded to the loss of most eggs in some hybrid families before the eyed-stage, and after which survival remained stable (Fig. S2). This indicates that most losses in hybrids are related to fertilisation failure or developmental deficiencies in early embryos. We observed an average fertilisation/survival rate reduction of 18.05% in PLxSB crosses compared to PLxPL crosses, and of 39% in SBxPL compared to SBxSB crosses.

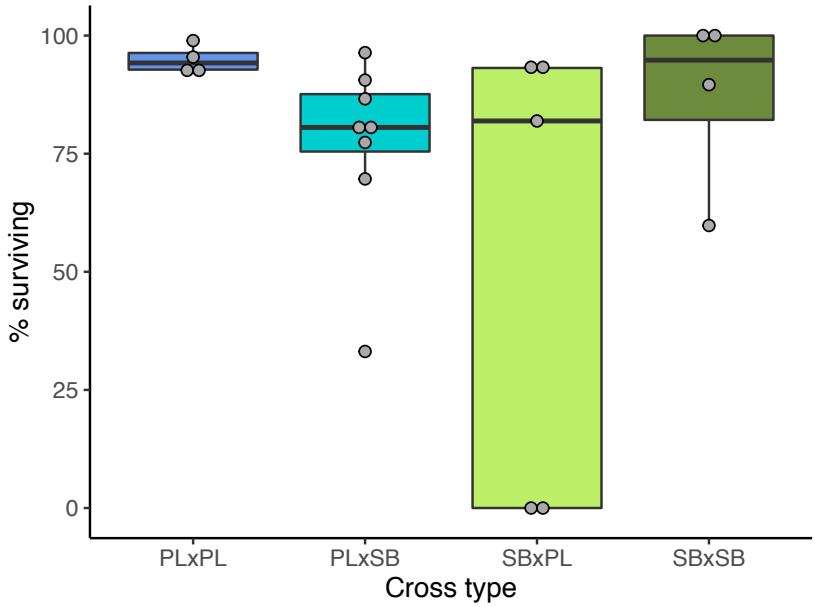


Fig. 3. Percentage of embryos successfully fertilized and surviving to hatching.

Phenotypic variations in juveniles.

Individuals from all cross types differed in body shape ten month after hatching (Fig 4, Table 2). SBxSB juveniles were deeper bodied (PC1 in Fig 4a) and a rounder snout with a lower, upward-pointing mouth compared to PLxPL juveniles (PC2 in Fig 4a). Juveniles from both hybrid cross types appeared to exhibit intermediate shapes (Fig 4b,c, Table S2), although a trend for a bias toward the maternal morph was observed in SBxPL hybrids (Fig 5). We also observed increase shape disparity in PLxSB specimens: the distribution of individuals spanned the range of values observed in all other cross types, and several specimens were located at higher values along PC1 and PC2 than SBxSB juveniles, *i.e*. showed "extreme benthic-like" features (Fige4b). This pattern was partially supported by pairwise comparisons reporting significant variance differences between SBxSB (lowest variance of all cross types) and PLxSB cross types (highest variance, Table S2).

We did not observe variations in growth amongst cross types from the month preceding the onset of exogeneous feeding to seven month later, *i.e*, ten months post-hatching (Fig. 5a). We also did not observe variations in any proxies of body condition among cross types at ten months post-hatching (Fig. 5b; Table S1).

Table 2. Nonparametric multivariate analysis of variance[1] of body shape in juvenile charr.

	d.f.	***R²***	***Z***	***P***
Age	4	0.04	3.75	<0.01
Log(size)	1	0.05	4.98	<0.01
Cross type	3	0.07	5.46	<0.01
Cross type × Family	9	0.10	5.51	<0.01
log(size) × Cross type	3	0.02	1.02	0.15
log(size) × Cross type × Family	13	0.05	0.72	0.24
Residuals	179	0.67		
Total	212			

[1]Based on a randomized premutation procedure with 10,000 iterations. Model formula: Procrustes coordinates ~ Age + log(size) + Cross type/Family + log(size) ´ Cross type/Family. Size: centroid size. Families are nested within cross types. Sequential sum or square: Type I.

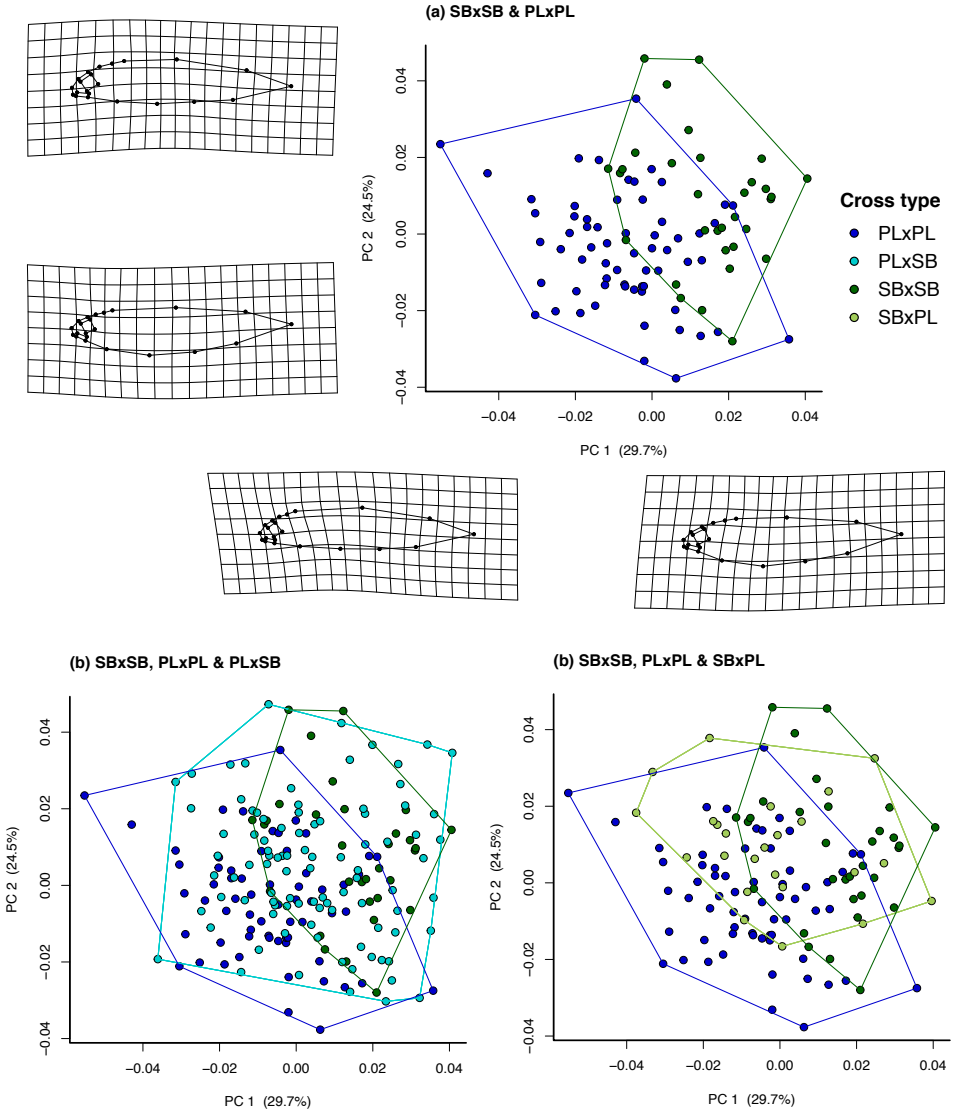


Fig. 4. Body shape variation among cross types. (a) Principal component (PC) plot of shape variations between the two pure-morph crosses. (b) Projection of PLxSB hybrids onto the first two eigenvectors of the PCA on pure morph crosses. (c) Projection of SBxPL hybrids onto the first two eigenvectors of the PCA on pure morph crosses. Convex hulls are for cross types. Transformation grids depict shape changes from the consensus landmark configuration at each extreme of the PC axes. The colour code is the same in all three panels.

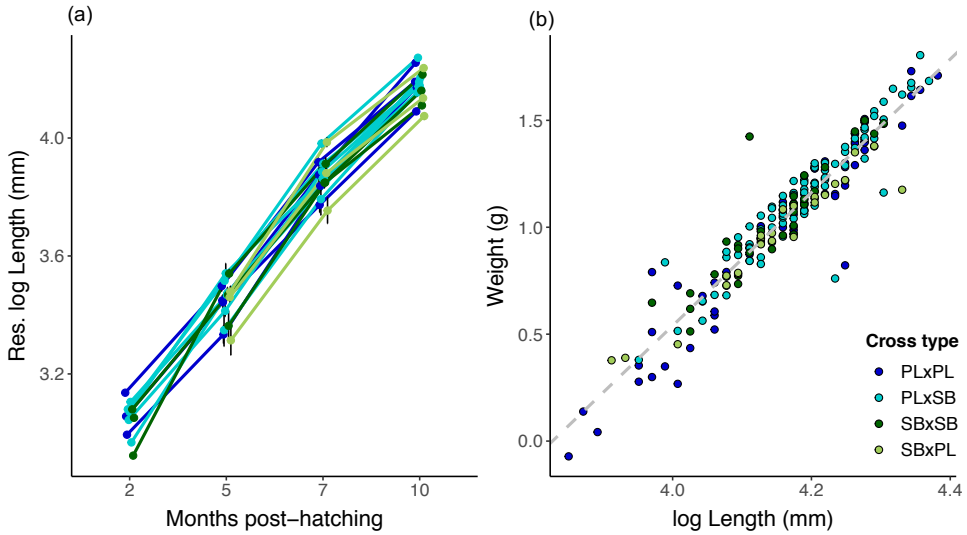


Fig. 5. Growth and body condition in charr juveniles of pure-morph and hybrid crosses. (a) Family mean (dot) and standard error (error bars) of late free embryos (2 months after hatching) and year-0 juveniles (5 to 10 months after hatching). (b) Length-weight regression as a proxy for body condition ten months after hatching. Dots are individuals from pooled families. The colour code is the same for both panels.

Reproductive isolation estimates.

RI estimates indicated that the PL-charr is almost completely reproductively isolated from SB-charr (Table 3). This strong RI is almost entirely generated by the demographic differences and the use of the spawning ground over time, which reduce the probability of gene flow to 5% at most (RI = 0.9 to 1.0). Consequently, assortative mating and postmating barriers, although moderate in absolute strength, had virtually no effects on RI in PL-charr.

RI was only partial in SB-charr. At Ólafsdráttur, the spawning ground where mature SB-charr occurred the most, the early spawning peaking reduced gene flow probability to 32% (RI = 0.36). Including the effect of assortative mating further reduced this probability to about 16% (total RI = 67). However, the demographic and the spatiotemporal use of the spawning ground at Mjóanes (were more PL-charr occur) induced a negative RI estimate, meaning that gene flow is higher than a in case of equal co-occurrence between morphs. Assortative mating re-establishes the probability of gene flow near the level expected under random mating (null RI).

When considering postmating isolation estimates in SB-charr (barrier to fertilisation and/or early embryo survival), we obtained a positive but weak total reproductive isolation estimate at Mjóanes (total RI = 0.10) and an estimate of strong total reproductive isolation at Ólafsdráttur (total RI = 0.78).

Table 3. Reproductive isolation (RI) estimates for each morph (PL- and SB-charr) at two spawning grounds (Mjóanes and Ólafsdráttur).

	Mjóanes				**Ólafsdráttur**			
	PL		**SB**		**PL**		**SB**	
	Absolute contribution	**Relative strength**	**Absolute contribution**	**Relative strength**	**Absolute contribution**	**Relative strength**	**Absolute contribution**	**Relative strength**
Premating isolation only								
Spatiotemporal isolation	0.72	0.81	-0.41	-	0.97	0.98	0.36	0.54
Assortative mating	0.17	0.19	0.40	-	0.02	0.02	0.30	0.46
Total RI	**0.90**		**-0.01**		**1.00**		**0.67**	
With postzygotic isolation								
Spatiotemporal isolation	0.72	0.79	-0.41	-	0.97	0.98	0.36	0.47
Assortative mating	0.17	0.19	0.40	-	0.02	0.02	0.30	0.39
Postmating isolation	0.02	0.02	0.24	-	0.00	0.00	0.12	0.15
Total RI	**0.92**		**0.10**		**1.00**		**0.78**	

Discussion.

Asymmetric reproductive isolation in PL- and SB-charr.

We obtained valuable cues of reproductive isolation in the sympatric PL- and SB-charr by combining fishing survey data, mate choice trails and common-garden experiment. PL-charr were completely reproductively isolated from SB-charr, demographic and temporal differences during spawning being virtually the only effective reproductive barrier. However, the strength of spatiotemporal barriers in SB-charr varied between the two studied sites from partial in Ólafsdráttur to negative in Mjóanes (where some SB-females were present throughout the spawning period but SB-males were heavily outnumbered by PL-males), which indicates a severe asymmetry in reproductive isolation. Yet, assortative mating appears to counteract these demographic effects, inducing moderate isolation in SB-charr at Ólafsdráttur, and no isolation at Mjóanes. Early post-mating barriers (*i.e.*, reducing fertilisation success and/or embryo survival) are also likely occurring in this system, and the cumulative effects of these barriers may generate modest isolation in SB-charr at Mjóanes where PL-charr dominate in numbers, and strong reproductive isolation in the other.The relevance of these estimates can be evaluated in light of available genome data. Applying the figure of 9.5% hybrids reported in Barchmann and colleagues' SNP Array data (Brachmann et al., 2021) to the Sobel and Chen's formula used in our study revealed a total reproductive isolation estimate of 0.81. This estimate is similar to those calculated with our data for PL-charr and for SB-charr at the Ólafsdráttur spawning site, but is much higher than our estimate for SB-charr at the Mjóanes site that is favoured by PL-charr. Note, however, that our estimates for premating barriers rely on the assumption that the fishing survey spans most the of spawning season. The spawning season of SB-charr may start from early September, so the total RI of SB-charr in may be underestimated.

Other barriers may also be at play in this system. Regarding pre-mating barrier, fertilisation failure may be underestimated as the synchrony of gamete release (which we by-passed during artificial fertilisation) is a crucial component of Arctic charr mating, modulating paternity and sperm competition, and being controlled by complex vibratory communication (Brattli, Egeland, Nordeide, & Folstad, 2018). Selection against hybrids might also reinforce reproductive isolation in SB-charr. Indeed, the average body shape in SBxPL hybrids differed from both PLxPL and PLxSB offspring, and were seemingly intermediate in mouth orientation and body depth. While we were not able to provide fitness estimates from our common-garden experiment, Franklin and colleagues reported lower growth rate in the wild in PL- and SB-charr with intermediate values for mouth position and body shape (Franklin & Morrissey, 2017), making the scenario of ecological postzygotic barrier plausible.

Evolutionary significance.

Understanding the rate of evolution of reproductive barriers along the speciation continuum is a primary goal in speciation research (Butlin et al., 2012; Matute & Cooper, 2021; Stankowski & Ravinet, 2021). A classical view holds that prezygotic barriers often evolve first (Matute & Cooper, 2021), and that,in populations under divergent selection, prezygotic barriers and extrinsic postzygotic isolation evolve rapidly, after which postzygotic isolation slowly builds-up (Seehausen et al., 2014). However, simulations on hybrid zones indicate

that prezygotic barriers may be ineffective in preventing gene flow early in speciation, and that at least some reduction in hybrid fitness is required to maintain reproductive isolation (Irwin, 2020). In the Arctic charr of Thingvallavatn, a recent (3500-4000 generations) as well as highly diverged system, we observed that while demographic differences and temporal mismatches in activities at spawning grounds constitute the most important prezygotic barrier, assortative mating can be an important barrier and selection against hybrids may also play a role. We did not observe direct evidence for intrinsic unviability – although the higher phenotypic variance in hybrids may underly developmental perturbations. SB- and PL-charr therefore appear to conform to the verbal model on the primary evolution of prezygotic/extrinsic postzygotic barriers. Note, however, that intrinsic postzygotic barriers may be commonplace in diverging salmonid with similar divergence time, as suggested by detrimental anomalies of bone development in F_1 hybrids from Arctic charr morphs of Sobachye Mountain Lake (Pichugin, 2009), extensive breakdown in gene regulation in F_2 hybrids of anadromous and resident brook charr, *Salvelinus fontinalis* (Mavarez et al., 2009), and developmental deficiencies in backcross and F_2 hybrids of whitefish morphs (Renaut & Bernatchez, 2011; Rogers & Bernatchez, 2006).

Comparable studies in postglacial freshwater fishes simultaneously estimating the relative importance of reproductive barriers – and using the commensurate RI estimates – are scarce. A notable exception involves a comprehensive study in stickleback fishes (Lackey & Boughman, 2017). This study reports that species pairs with similar divergence times as the PL- and SB-charr, exhibiting high reproductive isolation driven by prezygotic isolation only (lake/stream pairs and anadromous/freshwater pairs) or both by prezygotic and extrinsic postzygotic isolation (especially in a benthic/ limnetic morph pair), while intrinsic postzygotic barriers appeared only in a pair with much longer divergence time (Japan-Pacific pair with divergence occurring the 1.5 million years). Besides conforming to the "prezygotic barrier first" model, the similar patterns between benthic-limnetic stickleback pairs and the limnetic-benthic SB- and PL-charr suggest ecologically driven parallelisms in the evolution of reproductive barriers. Very interestingly, individual reproductive barriers were also asymmetric within early diverging stickleback pairs (as between PL- and SB-charr), but their cumulated effect resulted in symmetric reproductive isolation. While this may contrast with the PL-/SB-charr pair and indicate variations in the evolutionary paths to speciation, the postzygotic barriers that couldn't be included in our calculations might also make reproductive isolation in SB-charr matching the level observed in PL-charr. Another study on stickleback (although not providing comparable indices of reproductive isolation) reported no evidence for assortative mating between anadromous and freshwater morphs in River Tyne, Scotland, and suggested that selection against hybrids was the main barrier to gene flow (Jones, Brown, & Braithwaite, 2008). This study supports part of our results indicating that postzygotic isolation may evolve early in speciation and might not require prior evolution of premating isolation.

Besides northern freshwater fishes, comparative insight can also, to some extent, be gained from the multiple examples of adaptive radiation in cichlids. In these systems, prezygotic isolation evolves early during divergence, and hybrid inviability among young lineages is generally not observed – although some evidence for the early evolution of hybrid breakdown have been reported (Rometsch, Torres-Dowdall, & Meyer, 2020). This indicates a general trend for the primary evolution of prezygotic isolation, from which the Arctic charr of Thingvallavatn may be no exception.

Overall, our comprehensive study on reproductive isolation in sympatric Arctic charr morphs showed that spawning time is likely a primary source the isolation in a recent evolutionary system. However, assortative mating and postzygotic barriers may evolve early during speciation and be uncovered when the effects of temporal isolation become ineffective (as for the trends in SB-charr). More investigations are needed to establish the strengths of assortative mating and postmating isolation among PL- and SB-charr, as well as to locate these patterns among the general speciation trends. Surely, the patterns of reproductive isolation provided by our study (*i.e*, asymmetric and possibly multiple barriers) provides valuable insight for the comparative studies to come.

Acknowledgement.

Logistics and essential technical work behind the mating experiments were conducted together with Sindri Gíslason, Hanna María Kristjánsdóttir, Halldór P. Halldórsson, Hermann D. Guls, Sölvi R. Vignisson and Valdís Sigurðardóttir (Sudurnes Science and Learning Center). We thank Skúli Skúlason, Neil Metcalfe and Michael Morrissey for their theoretical guidance. The rearing experiments could be conducted thanks to the contributions of Bjarni K. Kristjánsson, Kári H. Árnason, Camille A. Leblanc, Rakel Þorbjörnsdóttir, and Christian Beuvard and David Benhaïm (Hólar University). We are also grateful to the Institute of Experimental Pathology at Keldur for offering rearing facilities. Field work was conducted with help from the Arctic Charr and Salmonids Group members (University of Iceland) and from Finnur Ingimarsson and Haraldur R. Ingvason (Natural History Museum of Kópavogur). We thank, Jóhann Jónsson and Rósa Jónsdóttir for allowing us to conduct research at their farm at Mjóanes, for offering facilities and for helping with field work. Much help for phenotyping was provided by Lieke Ponsioen, Sébastien L. T. Matlosz, Chloé Chavoix, and Alia Desclos.

Authors contributions.

Fishing surveys and gamete crossing: SSS, KHK, ZOJ and QJH. Embryo rearing: KHK, QJH. Designed the mate choice experiment: QJH, KHK. Conducted the mate choice experiment: QJH. Collected and preprocessed the mate choice experiment data and conducted exploratory analyses: RLKL. Collected morphological data in hybrids: CMR, MPS and RLKL. Collected data on specimens from the fishing survey: QJH, CCC, KHK. Conducted to a pilot experiment on assortative mating: QJH, KHK, CCC. Digitized and preprocessed the morphological data and conducted exploratory analyses: CMR. Analysed the data and wrote the manuscript: QJH. Critically revised the manuscript: KHK, SSS.

References.

Adams, D. C., & Otárola-Castillo, E. (2013). Geomorph: An R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution*, *4*(4), 393–399. https://doi.org/10.1111/2041-210X.12035

Adams, D. C., Rohlf, F. J., & Slice, D. E. (2013). A field comes of age: Geometric morphometrics in the 21st century. *Hystrix, 24*(1), 7–14. https://doi.org/10.4404/hystrix-24.1-6283

Berdan, E. L., & Fuller, R. C. (2012). A test for environmental effects on behavioral isolation in two species of killifish. *Evolution, 66*(10), 3224–3237. https://doi.org/10.1111/j.1558-5646.2011.01646.x

Bhat, S., Amundsen, P. A., Knudsen, R., Gjelland, K. Ø., Fevolden, S. E., Bernatchez, L., & Præbel, K. (2014). Speciation reversal in European whitefish (*Coregonus lavaretus* (L.)) caused by competitor invasion. *PLoS ONE*, 9(3), 1–10. https://doi.org/10.1371/journal.pone.0091208

Brachmann, M. K., Parsons, K., Skúlason, S., & Ferguson, M. M. (2021). The interaction of resource use and gene flow on the phenotypic divergence of benthic and pelagic morphs of Icelandic Arctic charr (*Salvelinus alpinus*). *Ecology and Evolution*, *11*(12), 1–20. https://doi.org/10.1002/ece3.7563

Brattli, M. B., Egeland, T. B., Nordeide, J. T., & Folstad, I. (2018). Spawning behavior of Arctic charr (*Salvelinus alpinus*): Spawning synchrony, vibrational communication, and mate guarding. *Ecology and Evolution, 17*(3), 8076–8087. https://doi.org/10.1002/ece3.4277

Butlin, R., Debelle, A., Kerth, C., Snook, R. R., Beukeboom, L. W., Castillo Cajas, R. F., … Schilthuizen, M. (2012). What do we need to know about speciation? *Trends in Ecology and Evolution, 27*(1), 27–39. https://doi.org/10.1016/j.tree.2011.09.002

Crespi, B. J., Nosil, P., Feder, J. L., de Carvalho, C. F., Villoutreix, R., Farkas, T. E., … Gompert, Z. (2018). Natural selection and the predictability of evolution in *Timema* stick insects. *Science, 359*(6377), 765–770. https://doi.org/10.1126/science.aap9125

De la Cámara, M., Ponsioen Lieke, Horta-Lacueva, Q. J. B., & Kapralova, K. H. (2021). The dynamic ontogenetic patterns of adaptive divergence and sexual dimorphism in Arctic charr. *BioRxiv*, 2021.01.15.426104. Retrieved from https://doi.org/10.1101/2021.01.15.426104

Doenz, C. J., Bittner, D., Vonlanthen, P., Wagner, C. E., & Seehausen, O. (2018). Rapid buildup of sympatric species diversity in Alpine whitefish. *Ecology and Evolution, 8*(18), 9398–9412. https://doi.org/10.1002/ece3.4375

Evgeny, E. V., Markev, Grigorii, N., & Pichugin, M. Y. (2018). Juvenile divergence in adaptive traits among seven sympatric fish eco-morphs arises before moving to different lacustrine habitats. *Journal of Evolutionary Biology*, 1–56. https://doi.org/10.1111/jeb.13283

Fabricius, E. (1953). Aquarium observations on the spawning behaviour of the char, *Salmo alpinus*. *Rep. Inst. Freshwater. Res. Drottningholm*, 37, 14–48.

Foote, A. D. (2017). Sympatric Speciation in the Genomic Era. *Trends in Ecology and Evolution, 33*(2), 85–95. https://doi.org/10.1016/j.tree.2017.11.003

Frandsen, F., Malmquist, H. J., & Snorrason, S. S. (1989). Ecological parasitology of polymorphic Arctic charr, *Salvelinus alpinus* (L.), in Thingvallavatn, Iceland. *Journal of Fish Biology, 34*(2), 281–297. https://doi.org/10.1111/j.1095-8649.1989.tb03309.x

Franklin, O. (2017). *Phenotypic selection of morphology and the role of trophic trade-offs in polymorphic Arctic charr (Salvelinus alpinus*). University of Guelph.

Franklin, O. D., & Morrissey, M. B. (2017). Inference of selection gradients using performance measures as fitness proxies. *Methods in Ecology and Evolution, 8*(6), 663–677. https://doi.org/10.1111/2041-210X.12737

Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution, 7*(11), 1325–1330. https://doi.org/10.1111/2041-210X.12584

García-Berthou, E. (2001). On the misuse of residuals in ecology: Regression of residuals vs. multiple regression. *Journal of Animal Ecology, 70*, 708–711. https://doi.org/10.1046/j.1365-2656.2002.00618.x

Guðbrandsson, J., Kapralova, K. H., Franzdóttir, S. R., Bergsveinsdóttir, T. M., Hafstað, V., Jónsson, Z. O., … Pálsson, A. (2019). Extensive genetic divergence between recently evolved sympatric Arctic charr morphs. *Ecology and Evolution, 9*(9), 1–20. https://doi.org/10.1101/489104

Hendry, A. P. (2009). Ecological speciation! Or the lack thereof? *Canadian Journal of Fisheries and Aquatic Sciences, 66*(8), 1383–1398. https://doi.org/10.1139/F09-074

Horta-Lacueva, Q. J. B., Benhaïm, D., Morrissey, M. B., Snorrason, S. S., & Kapralova, K. H. (2020). Animal personality adds complexity to the processes of adaptive divergence and speciation. *Animal Behaviour, 175*, 57–73. https://doi.org/10.1101/2020.03.02.971614

Horta-Lacueva, Q. J. B., Snorrason, S. S., Morrissey, M. B., Leblanc, C. A., & Kapralova, K. H. (2021). Multivariate analysis of morphology, behaviour, growth and developmental timing in hybrids brings new insights into the divergence of sympatric Arctic charr morphs. *BMC Ecology and Evolution, 21*(170), 1–15. https://doi.org/10.1186/s12862-021-01904-8

Horta-Lacueva, Q. J. -B., Ólafsdóttir, J. H., Finn, F., Fiskoviča, E., Ponsioen, L., Cámara, M., & Kapralova, K. H. (2022). From drones to bones: Assessing the importance of abiotic factors for salmonid spawning behaviour and embryonic development through a multidisciplinary approach. *Ecology of Freshwater Fish*, 1–11. https://doi.org/10.1111/eff.12654

Irwin, D. (2020). Assortative mating in hybrid zones is remarkably ineffective in promoting speciation. *The American Naturalist*, *195*(6), 1–51. https://doi.org/10.1086/708529

Jones, F. C., Brown, C., & Braithwaite, V. A. (2008). Lack of assortative mating between incipient species of stickleback from a hybrid zone. *Behaviour, 145*(4–5), 463–484. https://doi.org/10.1163/156853908792451520

Jonsson, B., Skúlason, S., Snorrason, S. S., Sandlund, O. T., Malmquist, H. J., Jónasson, P. M., … Lindem, T. (1988). Life history variation of polymorphic Arctic charr (*Salvelinus alpinus*) in Thingvallavatn, Iceland. *Canadian Journal of Fisheries and Aquatic Sciences, 45*(9), 1537-1547.

Kapralova, K. H. H., Morrissey, M. B. B., Kristjánsson, B. K. K., Lafsdóttir, G. Á., Snorrason, S. S. S., Ferguson, M. M. M., … Ferguson, M. M. M. (2011). Evolution of adaptive diversity and genetic connectivity in Arctic charr (*Salvelinus alpinus*) in Iceland. *Heredity, 106*(3), 472–487. https://doi.org/10.1038/hdy.2010.161

Kapralova, K. H., Jónsson, Z. O., Palsson, A., Franzdóttir, S. R., le Deuff, S., Kristjánsson, B. K., & Snorrason, S. S. (2015). Bones in motion: Ontogeny of craniofacial development in sympatric Arctic charr morphs. *Developmental Dynamics, 244*(9), 1168–1178. https://doi.org/10.1002/dvdy.24302

Kapralova, K. H., Morrissey, M. B., Kristjánsson, B. K., Olafsdóttir, G. Á., Snorrason, S. S., & Ferguson, M. M. (2011). Evolution of adaptive diversity and genetic connectivity in Arctic charr (*Salvelinus alpinus*) in Iceland. *Heredity, 106*(3), 472–487. https://doi.org/10.1038/hdy.2010.161

Lackey, A. C. R., & Boughman, J. W. (2017). Evolution of reproductive isolation in stickleback fish. *Evolution, 71*(2), 357–372. https://doi.org/10.1111/evo.13114

Lagunas, M., Pálssson, A., Jónsson, B., Jóhannsson, M., Jónsson, Z. Ó., Snorrason, S. S. (*in press*) Genetic structure and relatedness of brown trout (*Salmo trutta*) populations in the drainage basin of the Ölfusá river, South-West Iceland. *Peer J.*

Malmquist, A. H. J., Snorrason, S. S., Skúlason, S., Jonsson, B., Sandlund, O. T., & Jonasson, M. (1992). Diet differentiation in polymorphic Arctic charr in Thingvallavatn, Iceland. *Journal of Animal Ecology, 61*(1), 21–35.

Malmquist, H. J. (1992). Phenotype-specific feeding behaviour of two Arctic charr Salvelinus alpinus morphs. *Oecologia, 92*(3), 354–361.

Malmquist, Hilmar J., Ingimarsson, F., Ingvason, H. R., Stefánsson, S. M., & Hrafnsdóttir, Þ. (2020). Hlýnun Þingvallavatns og hitaferlar í vatninu. *Náttúrufræðingurinn, 90*(1), 80–99.

Matute, D. R., & Cooper, B. S. (2021). Comparative studies on speciation: 30 years since Coyne and Orr. *Evolution, 75*(4), 764–778. https://doi.org/10.1111/evo.14181

Mavarez, J., Audet, C., & Bernatchez, L. (2009). Major disruption of gene expression in hybrids between young sympatric anadromous and resident populations of brook charr

(*Salvelinus fontinalis* Mitchill). *Journal of Evolutionary Biology, 22*(8), 1708–1720. https://doi.org/10.1111/j.1420-9101.2009.01785.x

Nosil, P. (2012). The speciation continuum: what factors affect how far speciation proceeds? In *Ecological Speciation* (p. 208).

Ólafsdóttir, G. Á., Ritchie, M. G., & Snorrason, S. S. (2006). Positive assortative mating between recently described sympatric morphs of Icelandic sticklebacks. *Biology Letters, 2*(2), 250–252. https://doi.org/10.1098/rsbl.2006.0456

Olsen, A. M., & Westneat, M. W. (2015). StereoMorph: An R package for the collection of 3D landmarks and curves using a stereo camera set-up. *Methods in Ecology and Evolution, 6*(3), 351–356. https://doi.org/10.1111/2041-210X.12326

Østbye, K., Hagen Hassve, M., Peris Tamayo, A. M., Hagenlund, M., Vogler, T., & Præbel, K. (2020). "And if you gaze long into an abyss, the abyss gazes also into thee": four morphs of Arctic charr adapting to a depth gradient in Lake Tinnsjøen. *Evolutionary Applications*, *13*(6), 1240–1261. https://doi.org/10.1111/eva.12983

Parsons, K. J., Skúlason, S., & Ferguson, M. (2010). Morphological variation over ontogeny and environments in resource polymorphic arctic charr (*Salvelinus alpinus*). *Evolution and Development, 12*(3), 246–257. https://doi.org/10.1111/j.1525-142X.2010.00410.x

Pétursson, H. G., Norðdahl, H., & Ingólfsson. (2015). Late Weichselian history of relative sea level changes in Iceland during a collapse and subsequent retreat of marine based ice sheet. *Cuadernos de Investigación Geográfica, 41*(2), 261–277. https://doi.org/10.18172/cig.2741

Pichugin, M. Y. (2009). The development of an artificial hybrid and revealing elements of reproductive isolation between sympatric forms of Dryagin's char and *Salvelinus alpinus* complex (Salmonidae) from Sobachye Mountain Lake (Taimyr). *Journal of Ichthyology, 49*(3), 236–248. https://doi.org/10.1134/S0032945209030047

Ponsioen, L. (2020). *Reproductive barriers between sympatric morphs of Arctic charr (Salvelinus alpinus) in lake Thingvallavatn, Iceland*. University of Iceland.

Pounder, K. C., Mitchell, J. L., Thomson, J. S., Pottinger, T. G., & Sneddon, L. U. (2018). Physiological and behavioural evaluation of common anaesthesia practices in the rainbow trout. *Applied Animal Behaviour Science, 199*, 94–102. https://doi.org/10.1016/j.applanim.2017.10.014

Rabosky, D. L., & Matute, D. R. (2013). Macroevolutionary speciation rates are decoupled from the evolution of intrinsic reproductive isolation in *Drosophila* and birds. *Proceedings of the National Academy of Sciences of the United States of America, 110*(38), 15354–15359. https://doi.org/10.1073/pnas.1305529110

Ramsey, J., Bradshaw, H. D., & Schemske, D. W. (2003). Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution, 57*(7), 1520–1534. https://doi.org/10.2307/3448754

Renaut, S., & Bernatchez, L. (2011). Transcriptome-wide signature of hybrid breakdown associated with intrinsic reproductive isolation in lake whitefish species pairs (*Coregonus spp*. Salmonidae). *Heredity, 106*(6), 1003–1011. https://doi.org/10.1038/hdy.2010.149

Rogers, S. M., & Bernatchez, L. (2006). The genetic basis of intrinsic and extrinsic post-zygotic reproductive isolation jointly promoting speciation in the lake whitefish species complex (*Coregonus clupeaformis*). *Journal of Evolutionary Biology, 19*(6), 1979–1994. https://doi.org/10.1111/j.1420-9101.2006.01150.x

Rometsch, S. J., Torres-Dowdall, J., & Meyer, A. (2020). Evolutionary dynamics of pre-And postzygotic reproductive isolation in cichlid fishes: Reproductive isolation in cichlid fishes. *Philosophical Transactions of the Royal Society B: Biological Sciences, 375*(1806), 11–13. https://doi.org/10.1098/rstb.2019.0535

Sæmundsson, K. (1992). Geology of Thinvallavatn area. *Oikos, 64*, 40–68.

Salisbury, S. J., & Ruzzante, D. E. (2022). Genetic causes and consequences of sympatric morph divergence in Salmonidae: A Search for Mechanisms. *Annual Review of Animal Biosciences, 10*(1), 1–28. https://doi.org/10.1146/annurev-animal-051021-080709

Sandlund, O. T., Gunnarsson, K., Jónasson, P. M., Jonsson, B., Lindem, T., Magnússon, K. P., … Snorrason, S. S. (1992). The Arctic charr *Salvelinus alpinus* in Thingvallavatn. *Oikos, 64*(12), 305–351. https://doi.org/https://doi.org/10.2307/3545056

Sandlund, O. T., Jonsson, B., Malmquist, H. J., Gydemo, R., Lindem, T., Skúlason, S., … Jónasson, P. M. (1987). Habitat use of arctic charr *Salvelinus alpinus* in Thinvgallavatn, Iceland. *Environmental Biology of Fishes, 20*(4), 263–264.

Sandlund, O. T., Malmquist, H. J., Jonsson, B., Skúlason, S., Snorrason, S. S., Jónasson, P. M., … Lindem, T. (1988). Density, length distribution, and diet of age-0 Arctic charr Salvelinus alpinus in the surf zone of Thingvallavatn, Iceland. *Environmental Biology of Fishes, 23*(3), 183–195. https://doi.org/10.1007/BF00004909

Schluter, D. (1996). Ecological speciation in postglacial fishes. *Philosophical Transactions of the Royal Society B: Biological Sciences, 351*(1341), 807–814. https://doi.org/10.1098/rstb.1996.0075

Seehausen, O., Butlin, R. K., Keller, I., Wagner, C. E., Boughman, J. W., Hohenlohe, P. A., … Widmer, A. (2014). Genomics and the origin of species. *Nature Reviews Genetics, 15*(3), 176–192. https://doi.org/10.1038/nrg3644

Seehausen, O., & Wagner, C. E. (2014). Speciation in Freshwater Fishes. *Annual Review of Ecology, Evolution, and Systematics, 45*(1), 621–651. https://doi.org/10.1146/annurev-ecolsys-120213-091818

Sigurjónsdóttir, H., & Gunnarsson, A. (1989). Alternative mating tactics of arctic charr, *Salvelinus alpinus*, in Thingvallavatn, Iceland. *Environmental Biology of Fishes*, 26(3), 159–176. https://doi.org/10.1007/BF00004814

Skúlason, S., Noakes, D. L. G., & Snorrason, S. S. (1989). Ontogeny of trophic morphology in four sympatric morphs of Arctic charr *Salvelinus alpinus* in Thingvallavatn, Iceland. *Biological Journal of the Linnean Society, 38*(3), 281–301. https://doi.org/10.1111/j.1095-8312.1989.tb01579.x

Skúlason, S., Parsons, K. J., Svanbäck, R., Räsänen, K., Ferguson, M. M., Adams, C. E., … Snorrason, S. S. (2019). A way forward with eco evo devo: an extended theory of resource polymorphism with postglacial fishes as model systems. *Biological Reviews, 94*(5), 1786–1808. https://doi.org/10.1111/brv.12534

Skúlason, S., Snorrason, S. S., Noakes, D. L. G., Ferguson, M. M., & Malmquist, H. J. (1989). Segregation in spawning and early life history among polymorphic Arctic charr, *Salvelinus alpinus*, in Thingvallavatn, Iceland. *Journal of Fish Biology, 35*, 225–232. https://doi.org/10.1111/j.1095-8649.1989.tb03065.x

Skúlason, S., Snorrason, S. S., Ota, D., & Noakes, D. L. G. (1993). Genetically based differences in foraging behaviour among sympatric morphs of Arctic charr (Pisces: Salmonidae). *Animal Behaviour, 45*(6), 1179–1192. https://doi.org/https://doi.org/10.1006/anbe.1993.1140

Smith, T. B., & Skúlason, S. (1996). Evolutionary significance of resource polymorphisms in fishes , amphibians , and birds. *Annual Review of Ecology and Systematics, 27*(1), 111–133.

Snorrason, S.S., Skúlason, S., Sandlund, O. T., Malmquist, H. J., Jonsson, B., & Jónasson, P. M. (1989). Shape polymorphism in Arctic charr, *Salvelinus alpinus*, in Thingvallavatn, Iceland. *Physiolg Ecology Japan, 1*, 393–404.

Snorrason, Sigurður Sveinn, Skúlason, S., Jonsson, B., Malmquist, H. J., Jónasson, P. M., Sandlund, O. T., & Lindem, T. (1994). Trophic specialization in Arctic charr *Salvelinus alpinus* (Pisces; Salmonidae): morphological divergence and ontogenetic niche shifts. *Biological Journal of the Linnean Society, 52*(1), 1–18. https://doi.org/10.1111/j.1095-8312.1994.tb00975.x

Sobel, J. M., & Chen, G. F. (2014). Unification of methods for estimating the strength of reproductive isolation. *Evolution, 68*(5), 1511–1522. https://doi.org/10.1111/evo.12362

Stankowski, S., & Ravinet, M. (2021). Defining the speciation continuum. *Evolution, 75*(6), 1256–1273. https://doi.org/10.1111/evo.14215

Taylor, E. B., Boughman, J. W., Groenenboom, M., Sniatynski, M., Schluter, D., & Gow, J. L. (2006). Speciation in reverse: Morphological and genetic evidence of the collapse of a three-spined stickleback (*Gasterosteus aculeatus*) species pair. *Molecular Ecology, 15*(2), 343–355. https://doi.org/10.1111/j.1365-294X.2005.02794.x

Supplementary Figures and Tables.

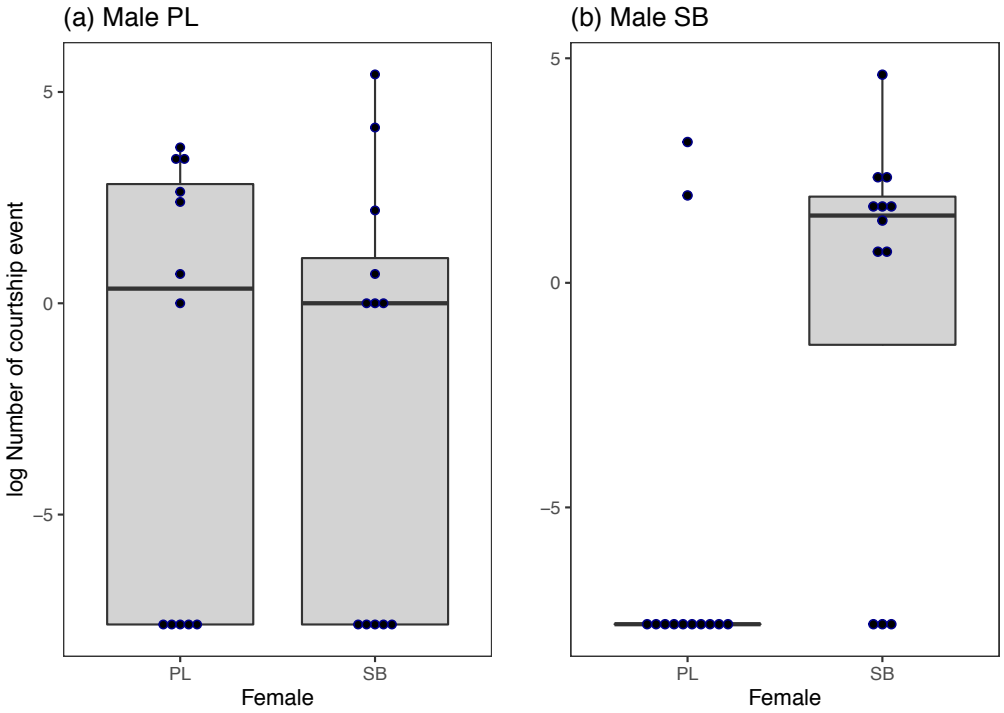


Fig. S1. Male courtship intensity toward females of each morph. Log-transformed count of courtship event by exhibited (a) PL-males and (b) SB-males.

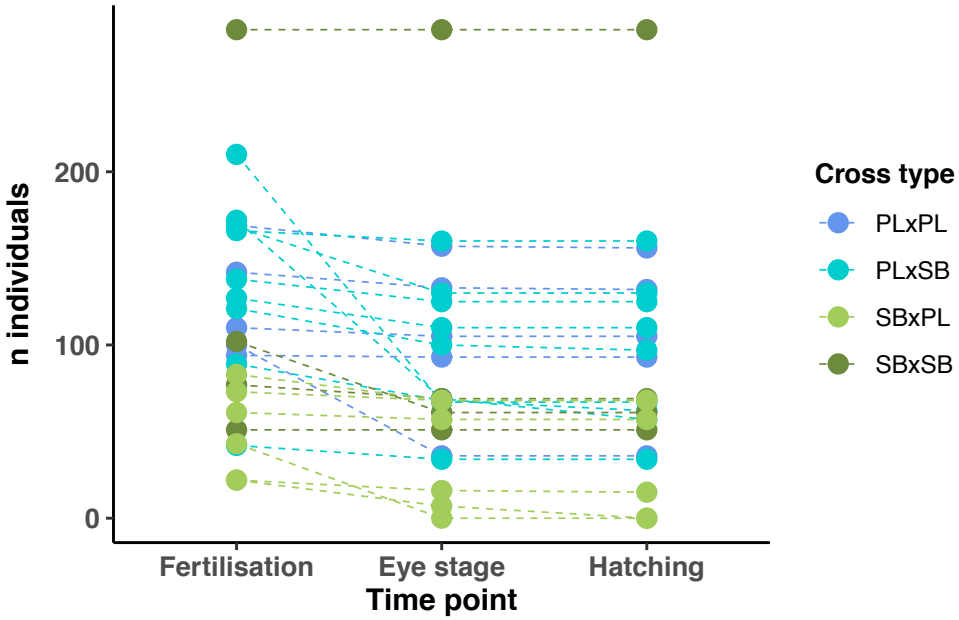


Fig. S2. Number of individuals in each family estimated at three developmental stages. Fertilisation: initial of number of eggs ; eye stage: embryos surviving to the completion of eye pigmentation ; hatching: free-living embryos on the week of hatching.

Table S1. Posterior mean and 95% Credible Intervals of the fixed effect of two models[1] for body conditions variations.

Response	Fixed effects	Posterior mean	95% Credible Intervals		
Weight/Length ratio	Density[2]	0.002	-0.002	;	0.006
	Cross PLxPL	-0.055	-0.131	;	0.019
	Cross PLxSB	-0.014	-0.069	;	0.060
	Cross SBxSB	0.011	-0.054	;	0.072
	Cross SBxPL	-0.059	-0.109	;	-0.007
Liver weight	Body weight	0.018	0.016	;	0.020
	Density[2]	0.000	-0.001	;	0.001
	Cross PLxPL	0.387	0.369	;	0.403
	Cross PLxSB	0.385	0.371	;	0.400
	Cross SBxSB	0.384	0.371	;	0.397
	Cross SBxPL	0.388	0.374	;	0.399

[1]Models formulae: Weight/Length ratio ~ Density + Cross type + Age (months), random effect= Family ; Wet liver weight (g) ~ Body weight (g) + Density + Cross + Age (months), random effect = Family.

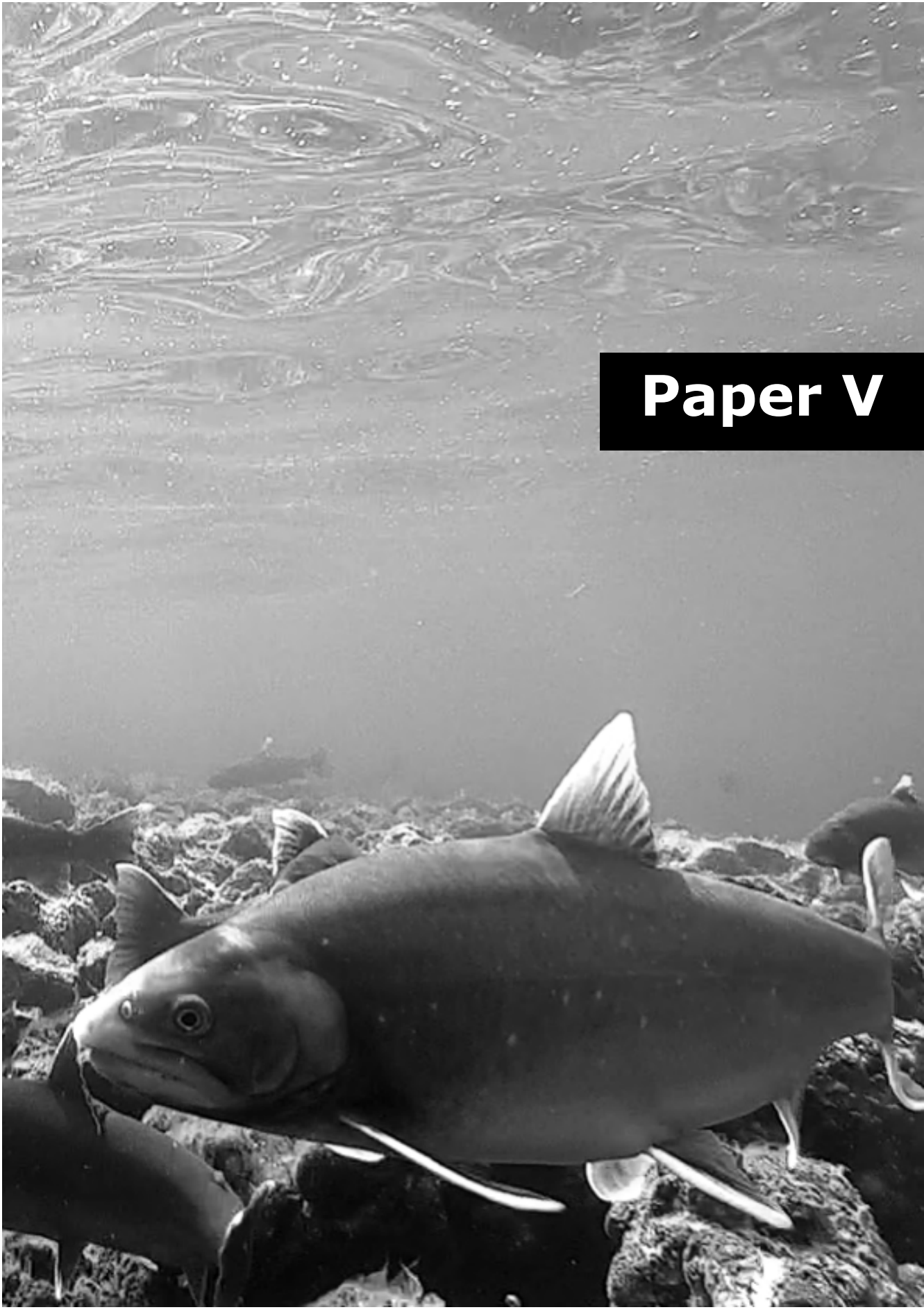
[2]Density: number of conspecifics in rearing bucket.

[3]Liver weight

Table S2. Attribute values, standardized score and *p*-value of the pairwise comparison of body shape among cross types, for average shape and phenotypic disparity[1].

	Mean			**Disparity**		
Comparison	*Dd*	*Z*	*P*	*Dd*	*Z*	*P*
PLxPLvs. PLxSB	$1.8e^{-02}$	7.08	<0.01	$9.5e^{-05}$	0.36	0.31
PLxPLvs. SBxSB	$2.5e^{-02}$	7.15	<0.01	$1.6e^{-04}$	0.81	0.20
PLxPLvs. SBxPL	$1.6e^{-02}$	1.99	0.04	$3.2e^{-06}$	-1.28	0.98
PLxSBvs. SBxSB	$1.1e^{-02}$	1.62	0.07	$2.5e^{-04}$	2.29	0.03
PLxSBvs. SBxPL	$1.8e^{-02}$	2.92	0.01	$9.8e^{-05}$	-0.08	0.46
SBxSBvs. SBxPL	$2.4e^{-02}$	3.81	<0.01	$1.5e^{-04}$	0.33	0.32

[1]Observed variances: PLxPL = 0.0008 ; SBxSB = 0.0006 ; PLxSB = 0.0009 ; SBxPL = 0.0008



Paper V

Received: 23 September 2021 | Revised: 16 December 2021 | Accepted: 27 January 2022

DOI: 10.1111/eff.12654

Ecology of FRESHWATER FISH WILEY

ORIGINAL ARTICLE

From drones to bones: Assessing the importance of abiotic factors for salmonid spawning behaviour and embryonic development through a multidisciplinary approach

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Funding information
The Icelandic Research Fund, Grant/Award Number: (173802-051 and 195964-051); Landsvirkjun and Palmi Jonsson's Nature Conservation Fund

Abstract

The ecology of salmonids is tightly linked to their spawning habitats, but the link between spawning site selection and phenology is poorly understood. To address this, we studied the Arctic charr (*Salvelinus alpinus*) from the postglacial lake Thingvallavatn (Iceland) through a multidisciplinary approach involving aerial surveys, behavioural observations, temperature monitoring and embryo rearing experiments. Aerial footage revealed that most nests (i.e. redds) were established in shallow parts of the spawning area, and we reported through direct observations trends for stronger male–male competition and for more frequent courtship behaviours in shallow than in deep redds. While water depth did not correlate with temperature at the time of spawning, the temperatures recorded at the shallow redds were consistently lower in the two months following the video recordings, likely because of the proximity of glacial outlets. Laboratory experiments demonstrated that the temperature regimes in shallow waters can delay hatching with about a month, likely impacting the phenology of the offspring. The viability of the Arctic charr in Thingvallavatn may thus depend on physical features like groundwater springs and upwelling water flows acting as "temperature shelters".

KEYWORDS
Arctic charr, development, habitat selection, salmonids, spawning behaviour, temperature

1 | INTRODUCTION

Salmonids are widespread over the northern hemisphere (Jonsson & Jonsson, 2009; Klemetsen, 2010; Quinn, 2018), but are affected by variations in abiotic conditions in many aspects of their biology and ecology (e.g. metabolism, migration patterns, breeding efforts (Enders & Boisclair, 2016; Jonsson & Jonsson, 2009; Kovach et al., 2013)). In these species, temperature is especially critical in early life stages, a period when mortality is the highest and the environmental conditions can drastically affect the performances of the fish later in life (Einum & Fleming, 2000; Jonsson & Jonsson, 2014; Metcalfe & Monaghan, 2001). Warmer than optimal temperatures may, for instance, not only increase direct mortality but also hasten hatching, decrease growth and yolk reabsorption, and influence the timing of emergence (Angilletta et al., 2008; Kelly et al., 2020; Quinn, 2018), which is thus likely to disrupt the synchrony between ontogenetic niche shifts and the seasonal availability of the offspring's prey (e.g. zooplankton, emerging chironomids, freshwater snails). Optimum abiotic conditions for developing embryos can be accessed through habitat selection. Thus, understanding how populations utilise

breeding locations in regard to varying physical parameters will help predicting how they may cope with future environmental changes.

Salmonids have evolved elaborate forms of habitat selection at the scale of micro-habitats within spawning grounds, probably in response to multiple cues. For example, bull trout (*Salvelinus confluentus*) select areas with high temperature and water discharge for spawning (Baxter & McPhail, 1999), female sockeye salmon (*Oncorhynchus nerka*) compete for deep breeding locations with low predation risk (Camacho & Hendry, 2020; Hendry et al., 2001) and brook charr (*Salvelinus fontinalis*) spawning sites are characterised by upwelling groundwater flow offering specific conditions of oxygen concentration and conductivity but no particular temperature regime (Guillemette et al., 2011). Thus, studying habitat selection in salmonids appears to be a tedious enterprise. However, intraspecific competition for spawning location is often fierce in this taxon (Auld et al., 2019; Esteve, 2005; Fleming, 1996; Quinn, 2018), which may provide some proxies for studying habitat selection. This competition generates complex mate choices (Auld et al., 2019; Dickerson et al., 2004) and elaborated male–male competition ruled by threatening displays and agonistic interactions (Esteve, 2005; Fabricius, 1953; Sigurjónsdóttir & Gunnarsson, 1989). Among the salmonids known for their complex reproductive structures, the large benthivorous (LB) charr of lake Thingvallavatn in Iceland is an emblematic study case. One of four morphs of Arctic charr within the lake, the LB charr is characterised by its large body size and its morphological adaptations to feed on benthic prey (Malmquist et al., 1992; Sandlund et al., 1992; Snorrason, Malmquist, et al., 1994; Snorrason, Skulason, et al., 1994). The breeding system of this morph is illustrative amongst iteroparous salmonids: a polygamous system composed of a large male (a "guarding male") defending a female hovering over her nest (a "redd"), while smaller males assume a "satellite" position and attempt to sneak fertilisation (Brattli et al., 2018; Esteve, 2005; Fleming, 1996; Sigurjónsdóttir & Gunnarsson, 1989). The LB charr have an unusually early spawning season in July–August as opposed to September–December for the other three morphs (Skúlason et al., 1989). This is believed to be linked to unique temperature conditions on the spawning grounds coupled with the seasonal burst of their main prey, adult snails *Radix peregra* (Skúlason et al., 1989; Snorrason, 2000). While multiple spawning locations have been described for the other three morphs of Arctic charr in the lake, there is only one known spawning area for the LB charr. The Ólafsdráttur area, located in the northeast part of the lake, is characterised by complex topology of the bottom and glacial springs scattered around the area, offering high spatial heterogeneity in physical parameters (Skúlason et al., 1989). The glacial springs are of particular interest as they provide constant temperatures of ca. 3°C in a lake commonly reaching 10–11°C over the thermocline in July–August (Jónasson, 1992). Together with the diurnal and easily observable breeding behaviour of the LB charr, these characteristics of their spawning grounds in Ólafsdráttur offer valuable opportunities for field research on habitat selection during spawning.

Here, we investigated how fish densities and mating behavioural can unravel the importance of temperature and/or location in regard with spawning habitat preferences in the LB charr. We collected aerial footage, video recording and continuous temperature measurements. By using female condition, male densities, aggression and courtship behaviours as proxies, we aimed at detecting spatial heterogeneity associated with differences in temperature and/or location of the redds and by extension unravel prime spawning sites within Ólafsdráttur. These data were used to address the following questions: (1) Is spawning of the LB charr in Ólafsdráttur segregated by location and/or by temperature? (2) Is redd temperature and/or location correlated with male densities or mating activities such as aggression and courtship? (3) What are the long-term temperature regimes (i.e. spanning the embryonic development) in the spawning grounds and how do they affect LB charr embryonic development?

2 | MATERIAL AND METHODS

2.1 | Study area

Thingvallavatn is an oligotrophic subarctic lake sitting in a graben of the Mid-Atlantic ridge in southwest Iceland (area of 84 km^2, mean depth: 34 m, maximum depth: 114 m). The present lake was formed following the last glacial retreat about 10,000 years ago (Pétursson et al., 2015). The physical structure of Thingvallavatn is characterised by a wide pelagic zone and three major benthic habitats: a 'stony littoral' zone (0–10 m deep) where the lava matrix is apparent, a densely vegetated zone of *Nitella opaca* algae (10–20 m deep) and a profundal zone (25 m and deeper) where the bottom is covered by a diatomic gyttja substrate (Snorrason, Skulason, et al., 1994). Thingvallavatn is a dimictic lake, commonly reaching 10–11°C in summer with a 2–3°C gradient over the thermocline (Adalsteinsson et al., 1992; Jónasson, 1992). The lake is 90% spring-fed with water percolating through lava, the main spring areas being located in the northern shores and exhibiting a constant temperature of 2.8–3.5°C (Jónasson, 1992). Warmer groundwater (7.5–10.7°C) also enters the south-western parts of the lake. The four morphs of Arctic charr (the planktivorous, the piscivorous, the large benthic and the small benthic) differ in habitat use, diet, head and body morphology, life history and parasitism (Jonsson et al., 1988; Skúlason et al., 1993; Snorrason, Malmquist, et al., 1994; Snorrason, Skulason, et al., 1994). The LB charr mostly occupies the stony littoral and the *Nitella* zones, where it forages on *R. peregra* snails. *R. peregra* abundant through the year, with a peak of adult availability in July. This burst of adult snails has been suggested to enable the unusually early gonad maturation of LB charr in late July while charr normally spawn in September–November (Snorrason, 2000).

2.2 | Estimating redd location and redd temperature during spawning

To assess spatial distribution of the LB redds within Ólafsdráttur, we took advantage of a behaviour displayed by female charr called

"clearing". When preparing for spawning, females clean the lava stones covering the shallow bottom of the lake from debris, silt and algae by repeatedly bending their bodies sideways. This activity creates dark spots called redds (i.e. salmonid nests) where spawning is occurring, making the location of incubating eggs easily detectable from the air (Figure 1).

To estimate the spatial distribution of the redds of LB charr, aerial photographs were taken over the Ólafsdráttur spawning area by flying a Mavic Pro drone (DJI, Shenzhen Dajiang Baiwang Technology) 50 m above the water surface level from the northern to the southern extremity of the main spawning ground (between 64°13'57.2"N 21°03'02.2"W and 64°13'51.4"N 21°03'15.8"W respectively). A composite image of the spawning ground (Figure S1) was then assembled with Image Composite Editor (Microsoft).

Video records of active redds were obtained from diving expeditions in Ólafsdráttur on August 8th–9th 2017, and on July 23rd–24th 2019. The redds were visually identified by two divers swimming along the shore from the northern to the southern extremity of the spawning ground. A redd was considered active when a female was observed hovering above it (Figure 1).

The behaviour of LB charr was video recorded at 18 active redds over a span of two non-consecutive years (Table 1). In 2019, the activities of all active redds observed in the spawning area were video recorded, whereas only five representative redds were selected in 2017. For each of the 18 video recordings, a GoPro camera (model 5 or 6) was placed at ca. 1.5 m from the edge of the redd. The depth of each redd was measured with a diving computer (Scubapro bottom timer–Digital 330 m; Figure S3). Each camera was left to record for a minimum of 45 min, after which we collected the material and marked the redd with a tagged anchor. During the 2019 filming, temperature was recorded every 15 min by placing a HOBO MX2202 logger at the edge of the redds.

2.3 | Studying the dynamics at the spawning grounds

Male density, female condition and a series of mating behaviours were used as proxies to address questions on habitat selection during LB spawning.

Male density was estimated by extracting frames from the video records on 60-second intervals. For each frame, we recorded the number of males present within two body lengths of the focal female, defined as the female residing at the redd at the start of filming (Figure 1). The ratio of body height (from the anterior extremity of the dorsal fin) to standard length (Figure 1b) was used for estimating female condition. Briefly, for each female, ten video frames showing the female swimming straight and perpendicularly to the camera were extracted, and body height and standard length measurements were taken in ImageJ (Schneider et al., 2012). The ICC (Intraclass Correlation Coefficient) of the index condition was high (ICC =0.73 [0.58–0.89]), meaning that variation in this index among photographs of the same female was much lower than the variation among females, and thus indicating low measurement error.

The intensity of courtship and aggression at each redd was estimated by sampling all occurrences of the relevant behaviours (Altmann, 1973). These behaviours belonged to ten subcategories previously described by Sigurjónsdóttir (1989) (Table 2). Behavioural events were sampled using BORIS v.7.8 (Friard & Gamba, 2016). Because male density is expected to increase shortly prior or after spawning event because of fertilisation sneaking or cannibalism (Frye et al., 2021; Rudolfsen et al., 2011), we tested whether variations in the frequency of spawning events affected our estimates of average aggression and male density. We did not observe trends among these variables (Figure S2).

FIGURE 1 Spawning LB charr. Main photo: aerial view of spawning redds (seen as darker spots with a female hovering above it) within in Ólafsdráttur spawning area. An example of a singular spawning redd is highlighted with a red circle. Inset: a videoframe used to estimate male densities and the ratio of body height (2) to standard length (1) in females

TABLE 1 Physical parameters and behavioural variables of the video-recorded redds

Redd ID	Depth category	Average Temperature (°C)	Video length (s)	Female condition	Average number of males	Freq. courtship events	Freq. aggression events	Temp logger collection date
2019_1	Deep	6.98	1430	0.22	1.00	0.28	0.39	July 23, 2019
2019_2	Shallow	6.98	1279	0.23	1.50	0.51	0.36	July 23, 2019
2019_4	Shallow	5.14	2586	0.24	2.82	0.64	1.54	July 23, 2019
2019_5	Shallow	5.66	841	0.28	3.40	1.72	3.51	July 23, 2019
2019_6	Shallow	5.32	3230	0.24	3.80	1.79	3.72	July 23, 2019
2019_7	Deep	7.68	5135	0.25	2.56	1.39	2.41	July 24, 2019
2019_8	Shallow	3.96	3099	0.23	3.22	2.05	5.15	July 24, 2019
2019_10	Shallow	7.93	3859	0.22	1.96	1.65	1.34	July 24, 2019
2019_11	Shallow	7.83	2316	0.24	4.59	1.82	5.04	Sept. 29, 2019
2019_12	Shallow	7.56	4626	0.25	1.91	2.99	3.02	Sept. 29, 2019
2019_17	Deep	8.16	4895	0.27	2.96	1.67	1.88	Sept. 29, 2019
2019_18	Deep	10.23	3428	0.25	1.11	1.06	0.63	Sept. 29, 2019
2019_91	Shallow	7.55	4112	0.25	2.91	1.76	3.25	Sept. 29, 2019
2019_92	Shallow	7.55	4093	NA	3.04	1.68	4.76	Sept. 29, 2019
2017_22	NA	NA	3133	0.26	2.53	1.68	4.19	NA
2017_23	NA	NA	4350	0.25	1.74	2.32	3.76	NA
2017_24	NA	NA	4275	0.28	2.40	1.46	5.15	NA
2017_25	NA	NA	3386	0.26	2.02	0.42	2.80	NA
2017_26	NA	NA	4599	0.25	1.96	2.03	5.23	NA

Several statistical methods have been advocated to deal with the modest sample sizes inherent to studies of wild, non-model organisms concerned with ethical constrains (Garamszegi, 2016). Because we faced this issue, we conducted our data analyses using two approaches: by direct graphical interpretation of raw data and by fitting models within a Bayesian framework, using the Markov Chain Monte Carlo (MCMC) methods. We tested for differences in male density, female condition, courtship intensity and aggression (response variables) in relation to (1) depth and (2) temperature. Two models were fitted per response variable, each model including either depth or the average temperature (at the time scale of the video recording) as a fixed effect. In our study, the redds were not spread evenly across depth, most being on a littoral "plateau" less than 1 m deep, while the remaining ones were located at 2 m or deeper. Therefore, and to conform with the rest of the results, depth was entered as a categorical variable: redds located between 0 and 1 m below the surface were categorised as "shallow" and redds deeper than 1 m were categorised as "deep". Behavioural variables were fitted with a Poisson distribution. We estimated the R^2 of these models following the methods for generalised linear models as described in Nakagawa and Schielzeth (2013).

We specified weakly informative priors (V_{res} =1, *nu* =0,002) and determined the quality of models by examining the trace plots, the posterior density plots and the effective sample sizes. The final number of MCMC iterations, thinning intervals and burn-in were 52,000; 400 and 120,000; respectively, apart for the models on density and depth (65,000; 500 and 150,000), and on behaviour and depth (91,000; 700 and 210,000). Inferences were made by comparing altogether the posterior modes, the value of a parameter that appeared the most after the resampling procedure, the 95% Credible Intervals (95% CrIS) and the posterior densities of the fixed effect estimates. These analyses were conducted with the R package MCMCglmm (Hadfield, 2010).

2.4 | Temperature and embryonic development

To obtain the temperature profiles of the redds over the first 2 months of the LB embryonic period, the temperature loggers were placed until September 29th in six redds, three of these were situated in shallow areas (0–2 m deep) and the remaining three in deeper areas (below 2 m). Temperature was recorded every 15 min using the same HOBO MX2202 loggers. The analyses were conducted in R (R Core Team, 2020).

Relative age of the embryos in deep and shallow redds were estimated from the temperature profiles using temperature units (TUs, the product of average daily temperature in degrees Celsius times the number of days post fertilisation (Quinn, 2018)) and tau-somite units (t_s). t_s are defined as the time it takes for one somite pair to form at a given temperature (Gorodilov, 1996). We characterised the variations in developmental rate between the two depth categories

TABLE 2 Description of the 11 aggression (4) and courtship (7) subcategories of the sampled behaviours

Category	Behaviour	Actions from the male	Actions from the female
Aggression	Attacking a satellite male	Any aggressive action, *i.e.*, chase, quick turn and display, chase with open mouth, biting towards a satellite male	Any aggressive action, *i.e.*, chase, quick turn and display, chase with open mouth, biting towards a satellite male
	Attacking a guarding male	Any aggressive action, *i.e.*, chase, quick turn and display, chase with open mouth, biting towards a guarding male	Any aggressive action, *i.e.*, chase, quick turn and display, chase with open mouth, biting towards a guarding male
	Circle display	Swimming in a circle with another individual, extending fins	Swimming in a circle with another individual, extending fins
	Attacking a female	Any aggressive action, *i.e.*, chase, quick turn and display, chase with open mouth, biting towards a female	–
Courtship	Lining up	Swimming up to the female and staying right next to her without quivering	Staying in the red and not swimming away when being approached by male
	Quivering	Quivering the whole body lined up against a female	Quivering the whole body next to a male in the redd
	Orgasm	Full body quivering, head stretched up, mouth open, back arched	Full body quivering, head stretched up, mouth open, back arched
	Orgasm with eggs	–	Same as orgasm but with visible eggs
	Orgasm with sperm	Same as orgasm but with visible sperm	–
	Quivering next to male	Male quivering against a male	–
	Digging	–	Laying on the side the female makes forceful movements from side to side to clean the substrate

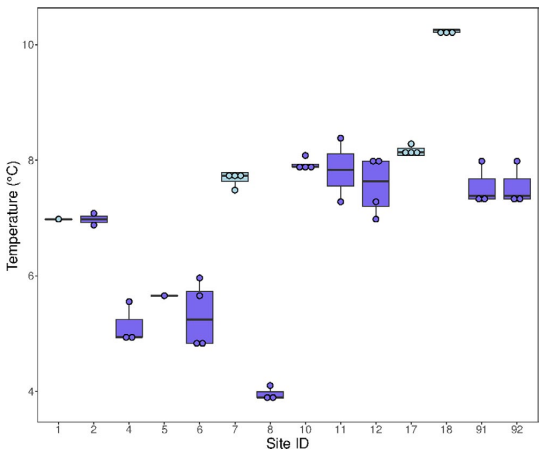

FIGURE 2 Box plot depicting the temperature measured at the redds during the video recordings. Light blue: shallow redds; dark blue: deep redds. Dots are individual temperature measurements

by using LB embryos raised in a common garden experiment. We sampled the LB embryos from the rearing setup and fixed them in 4% PFA at the developmental time points corresponding to the last day of temperature measurement at each depth category. We then visualised the stages of craniofacial development by staining the cartilage (alcian blue) and the bones (alizarin red) of the embryos following (Kapralova et al., 2015).

3 | RESULTS

3.1 | Redds' location and temperature during spawning

The redds were not evenly distributed over the spawning grounds, the majority of them (n = 81) were located in shallow areas while only a few (n = 17) were located in the deeper areas (composite image in Supporting information).

At the time of video recording, the average redd temperature of the three loggers in both depth categories ranged from 4.0 to 10.2°C (7.2 ± 1.6°C; mean ±standard). We did not observe patterns of temperature variation across depths at the time scale of the video records (Figure 2).

3.2 | Habitat selection of LB charr during spawning

We did not find evidence of variation in female height:length ratio (a proxy of body condition) across depth or temperature (Table 1, Table 3). Male density varied greatly among redds, with the average number of males per redd ranging between 1.0 and 4.6 (Table 1).

Test	Fixed effect	Posterior mode	95% CrI		
Condition and temperature	Intercept	−1.48	−1.65	–	−1.28
	Temperature	0.01	−0.02	–	0.03
Condition and depth	Intercept	−1.42	−1.47	–	−1.36
	Depth: deep	0.04	−0.06	–	0.12

TABLE 3 Posterior estimate at the fixed level, for the ratio of body height over standard length (as a proxy of body condition) across redd short-term temperatures and depth categories

TABLE 4 Posterior mode and 95% Credible intervals (95% CrI) of the fixed effects for the models testing female condition, temperature and depth on male density and male spawning behaviours

Test	Response variable	Fixed effect	Posterior mode	95% CrI		
Effect of female condition	Male density	Intercept	2.40	2.04	–	2.96
		Log(female condition)	0.00	−0.03	–	0.03
	Aggression	Intercept	3.09	2.21	–	3.81
		Log(female condition)	0.00	−0.03	–	0.03
	Courtship	Intercept	1.51	1.20	–	1.88
		Log(female condition)	0.00	−0.03	–	0.03
Effect of temperature	Male density	Intercept	1.61	−0.06	–	3.00
		Temperature	−0.09	−0.30	–	0.14
	Aggression	Intercept	2.03	0.17	–	3.55
		Temperature	−0.19	−0.41	–	0.07
	Courtship	Intercept	1.06	−1.24	–	2.72
		Temperature	−0.08	−0.32	–	0.24
	Female condition	Intercept	−1.48	−1.65	–	−1.28
		Temperature	0.01	−0.02	–	0.03
Effect of depth	Male density	Depth: shallow	1.12	0.66	–	1.39
		Depth: deep	0.70	0.27	–	1.22
	Aggression	Depth: shallow	1.06	0.71	–	1.49
		Depth: deep	0.14	−0.75	–	1.08
	Courtship	Depth: shallow	0.52	0.20	–	1.07
		Depth: deep	0.04	−0.62	–	0.69
	Female condition	Intercept	−1.42	−1.47	–	−1.37
		Depth: deep	0.03	−0.06	–	0.11

Male density appeared to be stable in each redd over the time period of the video recordings (Figure S2). We did not observe correlations between male density and female condition, nor did we observe trends involving female condition and aggression or courtship (Table 3). Combined, these results suggest that females with different body condition may have an equal access to redd location. Furthermore, males may not be competing for females based on their body condition.

Male densities tended to be lower in the deep redds than in the shallow redds (Table 4, Figure 3). Furthermore, aggression appeared to be higher in shallow redds, although this trend comes with a relatively low sample size and high estimate uncertainties (Table 4, Figure 4). Depth appeared to explain a large amount of the variation in aggression, although the R^2 posterior estimates are bounded with high uncertainty (R^2 =0.52 [0.00–0.75], posterior mode [95% CrI]). We also observed a slight trend for more intense courtship in shallow redds (Table 4, Figure 4), but a low amount of variation in these behaviours tended to be explained by depth (R^2 = .00 [0.00–0.60], posterior mode [95% CrI]).

Contrary to our predictions, we did not observe correlations between temperature and any variable characterising male spawning behaviour (density, aggression and courtship) (Table 4).

3.3 | Temperature and embryonic development

While temperature variations across depths were not detected during the time of video recording, the shallow and deep redds showed stable and distinct temperature profiles in the two months following the video recordings (Figure 5a): the average temperature recorded

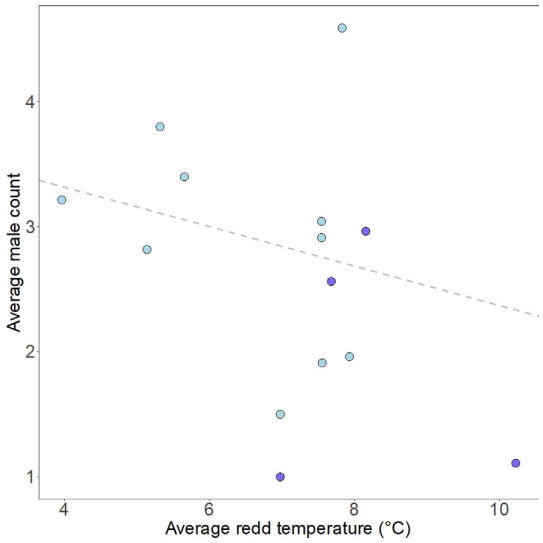

FIGURE 3 Male counts over short-term temperature (averaged over the filming time period). The dashed line represents the estimates of the effects of temperature from a linear model including depth and temperatures ($R^2 = .17$)

at the deep redds was 5.2°C, whereas the average temperature of the shallow redds was 3.7°C (Figure 5a).

In salmonids, developmental rate is a function of temperature (i.e. embryos reared in higher temperature will develop faster than embryos reared in lower temperatures), so developmental time points are expressed here in TUs. Embryos resulting from fertilisation on the day of filming (July 26–27th) from shallow redds would have reached about 268 TUs by on the last day of temperature measurement (September 29th), whereas embryos from deeper redds would have reached about 380 TUs (Figure 5b). By using tausomite units (t_s) to assess developmental stages in anatomical terns (Gorodilov, 1996), we estimated that these two values of TUs corresponded to the same pre-hatching stage. However, LB embryos raised in our common garden setup differ largely in their craniofacial development embryos at these two time-points: while at 268 TUs ($170t_s$) chondrogenesis is in its early stages and most craniofacial elements are still being formed, at 380 TUs ($235t_s$) this process is almost complete, and ossification is starting to take place (Figure 5c).

4 | DISCUSSION

Combining aerial observations, temperature monitoring and behavioural sampling uncovered compelling characteristics of the spawning strategies of the large benthic charr of Thingvallavatn. First, spawning efforts appeared to target a restricted shallow area of the spawning grounds, as revealed by the higher redd density observed from aerial photographs and by trends for higher intensity of aggression and courtship behaviours over shallow redds. Second, the redds located in shallow areas appeared to be under a different temperature regime than the redds located in deeper parts of the spawning grounds. While we did not report evidence for temperature differences across depth during the spawning season, the shallow redds appeared to progressively cool down after the end of the spawning season. Temperatures in the shallow areas remained constantly lower over the next couple of months when compared to temperatures in the deeper areas, which would have inevitably affected embryo developmental rates.

By extrapolating the mean temperature of the last ten recorded days to the next two months, we estimated that the embryos incubating in deep redds would hatch approximately a month earlier (ca. 35–40 days) than their conspecifics from the shallow redds. The same extrapolation suggests that embryos growing in deep redds would start feeding one and a half to two months earlier (ca. 50 days) than their conspecifics growing in the shallow redds. These estimates, however, need to be taken with caution since the effects of temperature on development are not linear and can vary across ontogenetic stages (Cook et al., 2018; Jeuthe et al., 2016; Marr, 1966). In the Arctic charr, for example, a first warmer incubation period of less than a week, followed by the cold ambient temperatures for the species (2.3°C) seems to increase survival and decrease spinal deformities compared to a constant cold treatment (Jeuthe et al., 2016). Furthermore, growth and the metabolic responses to temperature often vary among salmonid populations (Cook et al., 2018; Jonsson & Jonsson, 2009).

Despite the complex effects of temperature on development, it is undeniable that the observed variations have substantial consequences on the ecology of LB charr juveniles. The spring-fed site of Ólafsdráttur provides colder temperatures in the autumn but warmer temperatures in the winter compared to Svínanes, the best-known spawning ground of the other three Arctic charr morphs (Skúlason et al., 1989). The temperature regime of Ólafsdráttur might also make chironomid larvae and pupae available for a longer time in the winter, as indicated by stomach content studies of age-0 charr (Sandlund et al., 1988). These ecological factors may enable both the early spawning of LB charr and the fast growth of their juveniles (Skúlason et al., 1989). This remarkable spawning strategy is believed to be an adaptation to the life cycle of the LB carr main prey, *Radix peregra*, which is available all year-round with a peak of adult density in May–June (Snorrason, 2000). The higher number of redds in the shallow parts of Ólafsdráttur suggest that juveniles growing there may benefit from the best of these ecological parameters, and that habitat selection may have evolved accordingly in the LB charr. However, one needs to apply caution to such interpretations. First, other confounding effects than temperature might be in play, like the proximity of shallow areas to the surf zone towards which the offspring is believed to migrate to forage (Sandlund et al., 1988). Second, even if the ecological conditions enounced above vary with depth, mechanisms like growth compensation might reduce their significance for offspring development. Yet, these compensation mechanisms are often costly later in life (Metcalfe & Monaghan, 2001).

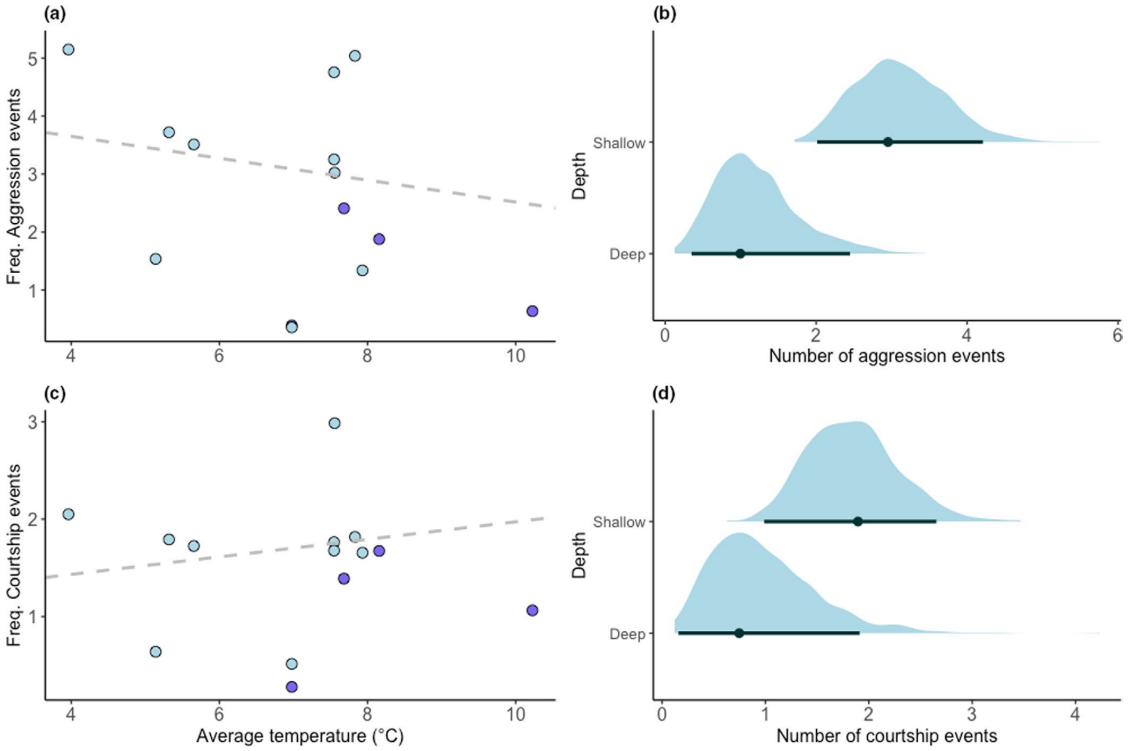

FIGURE 4 Intensity of aggression and courtship displays across temperature and depth. (a-c) Frequency of (a) aggression events and (b) courtship display. Dashed line: estimates the effect temperature, from a linear model including depth and temperature as fixed effects. (b-c) Posterior densities, posterior modes and 95% credible intervals for the fixed effects of the model about (b) aggression and (d) courtship

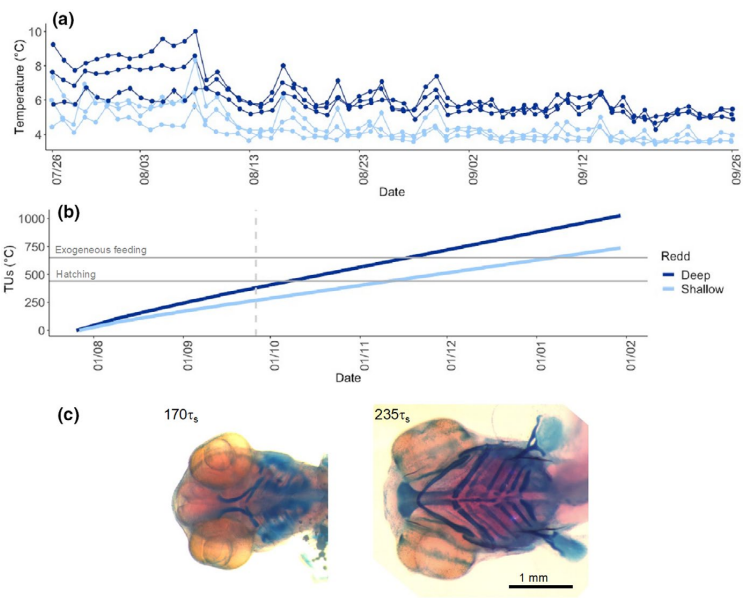

FIGURE 5 Temperatures at six redds (three shallow and three deep redds) and putative consequences on the embryos. (a) Mean daily temperatures over 2 months after spawning (error bars: standard error). (b) Estimated developmental time-points in temperature units (TUs) based on the mean daily temperatures. Vertical dashed line: last day of temperature measurement, after which the temperatures are extrapolated as the mean over the last 8 days of measurements. Horizontal lines: estimated dates of hatching (bottom) and of the onset of exogeneous feeding (top) according to common garden observations. (c) Stained LB charr embryos at estimated in shallow (left) and deep redds (right), ca. $170t_s$ and $235t_s$ respectively

The apparent preference for colder shallow waters of the LB charr in Thingvallavatn prompts questions on LB charr conservation. Arctic charr embryos have very low temperature tolerance, and warming temperatures have already been identified as a likely factor for the decline in southern populations (Baroudy & Elliott, 1994; Gerdeaux, 2011). In Thingvallavatn, the site of Ólafsdráttur provides

stable temperature conditions through the season compared to the spawning sites utilised by the other charr morphs (Skúlason et al., 1989). Because the groundwater responsible for the temperature variations in Ólafsdráttur originates from the Langjökull ice cap (Jónasson, 1992), one may expect this particular spawning area to act like a nursing refuge for the Arctic charr in the face of warming surface temperature. However, negative mass changes are being observed in the Langjökull ice cap (Foresta et al., 2016), and the long-term integrity of the glacial outlets remains unknown.

Furthermore, our most striking observation complexify our view on how the Arctic charr of Thingvallavatn may cope with either of these scenarios: that the charr favour spawning habitats with seasonal temperature differences not observable during the breeding season. The fishes might, therefore, rely on indirect environmental cues. This might be detrimental in a context of environmental changes if the loss of correlation between such cues and later temperature regimes leads to the selection of habitats with inappropriate developmental conditions. In such scenario, the LB charr may face a "developmental trap" (Van Dyck et al., 2015). Optimistically, the Arctic charr might rely on the groundwater flow as a cue in itself. Groundwater springs and water upwelling are believed to be of prime importance in the recent evolutionary history of charr by constituting thermal regia in periglacial environments (Power, 2002). Groundwater flow has also been documented often – though not always – linked to habitat selection in several salmonid species, especially in charr (Blanchfield & Ridgway, 2005; Curry et al., 1995; Guillemette et al., 2011). The potential of groundwater to buffer air temperature changes depends on many geographical and the topographical factors, such as vegetation cover, depth and infiltration rate, which complicates prediction about their quality as thermal refugia for spawning (Meisner et al., 1988). Furthermore, the effects of groundwater flow on the persistence of salmonid populations can be very complex. For example, the brook charr from Rock Lake (Adirondack Mountains, USA) spawn on groundwater springs with constant temperature, but increased summer air temperature delays spawning time, potentially worsening the asynchrony between offspring and prey emergence (Warren et al., 2012). Thus, the survival of the LB charr and other Arctic charr populations may depend not only on the persistence of groundwater outlets providing consistent temperature regimes, but also on many other ecological and evolutionary factors, such as the population specific ability to track changes in the distribution and the availability of these habitats.

ACKNOWLEDGEMENTS

This study greatly benefited from the intellectual input of Sigurður Snorasson. We thank Skúli Skúlason, Neil Metcalfe, Michael Morissey and Hrefna Sigurjónsdóttir for their advice on multiple aspects of the project. We are also grateful to Rebecca Lesdalon, Cécile Rayssac, Marie Sciannamea, Dagný Rúnnasdóttir and Sylvain Moinard for their help during fieldwork. This work was achieved thanks to the compliance of Thingvellir National Park, the help from the "Arctic charr and Salmonids" research group for producing the embryos, and the support of the Department of Aquaculture and Fish Biology at Hólar University for hosting and rearing the embryos. Lastly, we thank three anonymous reviewers for their helpful comments.

AUTHOR CONTRIBUTIONS

Conceived and designed the investigation: JO, KK and QH. Performed field and/or laboratory work: JO, KK, LP and QH. Analysed the data: EF, FF, QH and MC. Contributed materials, reagents and/or analysis tools: KK and JO. Wrote the paper: KK, MC, QH and LP. All authors critically revised the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The aerial footage and the datasets are available in the supporting information (datqset.zip). R code is available on https://github.com/quentin-evo/LB-temperature/blob/main/LB-spawning-script.md.

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REFERENCES

Adalsteinsson, H., Jónasson, P. M., & Rist, S. (1992). Physical characteristics of Thingvallatn, Iceland. *Oikos*, *64*, 121–135.

Altmann, J. (1973). Observtional study of behavior: Sampling methods. *Behaviour*, *49*, 227–266.

Angilletta, M. J., Ashley Steel, E., Bartz, K. K., Kingsolver, J. G., Scheuerell, M. D., Beckman, B. R., & Crozier, L. G. (2008). Big dams and salmon evolution: Changes in thermal regimes and their potential evolutionary consequences. *Evolutionary Applications*, *1*, 286–299.

Auld, H. L., Noakes, D. L. G., & Banks, M. A. (2019). Advancing mate choice studies in salmonids. *Reviews in Fish Biology and Fisheries*, *29*, 249–276.

Baroudy, E., & Elliott, J. M. (1994). The critical thermal limits for juvenile Arctic charr *Salvelinus alpinus*. *Journal of Fish Biology*, *45*, 1041–1053.

Baxter, J. S., & McPhail, J. D. (1999). The influence of redd site selection, groundwater upwelling, and over-winter incubation temperature on survival of bull trout (Salvelinus confluentus) from egg to alevin. *Canadian Journal of Zoology*, *77*, 1233–1239.

Blanchfield, P. J., & Ridgway, M. S. (2005). The relative influence of breeding competition and habitat quality on female reproductive success in lacustrine brook trout (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences*, *62*, 2694–2705.

Brattli, M. B., Egeland, T. B., Nordeide, J. T., & Folstad, I. (2018). Spawning behavior of Arctic charr (*Salvelinus alpinus*): Spawning synchrony, vibrational communication, and mate guarding. *Ecology and Evolution*, *17*, 8076–8087.

Camacho, C., & Hendry, A. P. (2020). Matching habitat choice: it's not for everyone. *Oikos*, *129*, 689–699. https://doi.org/10.1111/oik.06932

Cook, C. J., Burness, G., & Wilson, C. C. (2018). Metabolic rates of embryos and alevin from a cold-adapted salmonid differ with

temperature, population and family of origin: Implications for coping with climate change. *Conservation Physiology*, *6*, 1–16. https://doi.org/10.1093/conphys/cox076

Curry, R. A., Noakes, D. L. G., & Morgan, G. E. (1995). Groundwater and the incubation and emergence of brook trout (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences*, *52*, 1741–1749.

Dickerson, B. R., Willson, M. F., Bentzen, P., & Quinn, T. P. (2004). Size-assortative mating in salmonids: Negative evidence for pink salmon in natural conditions. *Animal Behaviour*, *68*, 381–385.

Einum, S., & Fleming, I. A. (2000). Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). *Evolution*, *54*, 628–639.

Enders, E. C., & Boisclair, D. (2016). Effects of environmental fluctuations on fish metabolism: Atlantic salmon *Salmo salar* as a case study. *Journal of Fish Biology*, *88*, 344–358.

Esteve, M. (2005). Observations of spawning behaviour in Salmoninae: *Salmo*, *Oncorhynchus* and *Salvelinus*. *Reviews in Fish Biology and Fisheries*, *15*, 1–21.

Fabricius, E. (1953). Aquarium Observations on the Spawning Behaviour of the Char, *Salmo alpinus*. *Rep. Inst. Freshwater. Res. Drottningholm*, *37*, 14–48.

Fleming, I. A. (1996). Reproductive strategies of Atlantic salmon: Ecology and evolution. *Reviews in Fish Biology and Fisheries*, *6*, 379–416.

Foresta, L., Gourmelen, N., Pálsson, F., Nienow, P., Björnsson, H., & Shepherd, A. (2016). Surface elevation change and mass balance of Icelandic ice caps derived from swath mode CryoSat-2 altimetry. *Geophysical Research Letters*, *43*, 12138–12145.

Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, *7*, 1325–1330.

Frye, M., Egeland, T. B., Nordeide, J. T., & Folstad, I. (2021). Cannibalism and protective behavior of eggs in Arctic charr (*Salvelinus alpinus*). *Ecology and Evolution*, *11*, 14383–14391.

Garamszegi, L. Z. (2016). A simple statistical guide for the analysis of behaviour when data are constrained due to practical or ethical reasons. *Animal Behaviour*, *120*, 223–234.

Gerdeaux, D. (2011). Does global warming threaten the dynamics of Arctic charr in Lake Geneva? *Hydrobiologia*, *660*, 69–78.

Gorodilov, Y. N. (1996). Description of the early ontogeny of the Atlantic salmon, *Salmo salar*, with a novel system of interval (state) identification Yuriy. *Environmental Biology of Fishes*, *47*, 109–127.

Guðbrandsson, J., Kapralova, K. H., Franzdóttir, S. R., Bergsveinsdóttir, T. M., Hafstað, V., Jónsson, Z. O., Snorrason, S. S., & Pálsson, A. (2019). Extensive genetic divergence between recently evolved sympatric Arctic charr morphs. *Ecology and Evolution*, *9*, 1–20.

Guillemette, F., Vallée, C., Bertolo, A., & Magnan, P. (2011). The evolution of redd site selection in brook charr in different environments: Same cue, same benefit for fitness. *Freshwater Biology*, *56*, 1017–1029.

Hadfield, J. D. (2010). MCMCglmm: MCMC methods for multi-response GLMMs in R. *Journal of Statistical Software*, *33*, 1–22.

Hendry, A. P., Berg, O. K., & Quinn, T. P. (2001). Breeding location choice in salmon: Causes (habitat, competition, body size, energy stores) and consequences (life span, energy stores). *Oikos*, *93*, 407–418. https://doi.org/10.1034/j.1600-0706.2001.930306.x

Jeuthe, H., Brännäs, E., & Nilsson, J. (2016). Effects of variable egg incubation temperatures on the embryonic development in Arctic charr *Salvelinus alpinus*. *Aquaculture Research*, *47*, 3753–3764.

Jónasson, P. M. (1992). The ecosystem of Thingvallavatn: A synthesis. *Oikos*, *64*, 405–434.

Jonsson, B., & Jonsson, N. (2009). A review of the likely effects of climate change on anadromous Atlantic salmon *Salmo salar* and brown trout *Salmo trutta*, with particular reference to water temperature and flow. *Journal of Fish Biology*, *75*, 2381–2447.

Jonsson, B., & Jonsson, N. (2014). Early environment influences later performance in fishes. *Journal of Fish Biology*, *85*, 151–188.

Jonsson, B., Skúlason, S., Snorrason, S. S., Sandlund, O. T., Malmquist, H. J., Jónasson, P. M., Gydemo, R., & Lindem, T. (1988). Life history variation of polymorphic Arctic charr (*Salvelinus alpinus*) in Thingvallavatn, Iceland. *Canadian Journal of Fisheries and Aquatic Sciences*, *45*, 1537–1547.

Kapralova, K. H., Franzdóttir, S. R., Jónsson, H., Snorrason, S. S., & Jónsson, Z. O. (2014). Patterns of MiRNA expression in Arctic charr development. *PLoS One*, *9*(8), e106084. https://doi.org/10.1371/journal.pone.0106084

Kapralova, K. H., Jónsson, Z. O., Palsson, A., Franzdóttir, S. R., le Deuff, S., Kristjánsson, B. K., & Snorrason, S. S. (2015). Bones in motion: Ontogeny of craniofacial development in sympatric Arctic charr morphs. *Developmental Dynamics*, *244*, 1168–1178.

Kelly, S., Moore, T. N., de Eyto, E., Dillane, M., Goulon, C., Guillard, J., Lasne, E., McGinnity, P., Poole, R., Winfield, I. J., Woolway, R. I., & Jennings, E. (2020). Warming winters threaten peripheral Arctic charr populations of Europe. *Climatic Change*, *163*, 599–618.

Klemetsen, A. (2010). The charr problem revisited: exceptional phenotypic plasticity promotes ecological speciation in postglacial lakes. *Freshwater Reviews*, *3*, 49–74. https://doi.org/10.1608/FRJ-3.1.3

Kovach, R. P., Joyce, J. E., Echave, J. D., Lindberg, M. S., & Tallmon, D. A. (2013). Earlier migration timing, decreasing phenotypic variation, and biocomplexity in multiple salmonid species. *P LOS ONE*, *8*(1), e53807.

Malmquist, A. H. J., Snorrason, S. S., Skúlason, S., Jonsson, B., Sandlund, O. T., & Jonasson, M. (1992). Diet Differentiation in polymorphic Arctic charr in Thingvallavatn, Iceland. *Journal of Animal Ecology*, *61*, 21–35.

Marr, D. H. A. (1966). Influence of temperature on the efficiency of growth of salmonid embryos. *Nature*, *212*, 957–959.

Meisner, J. D., Rosenfeld, J. S., & Regier, H. A. (1988). The role of groundwater in the impact of climate warming on stream Salmonines. *Fisheries*, *13*, 2–8.

Metcalfe, N. B., & Monaghan, P. (2001). Compensation for a bad start: grow now, pay later? *Trends in Ecology & Evolution*, *16*, 254–260.

Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *4*, 133–142.

Pétursson, H. G., Norðdahl, H., & Ingólfsson. (2015). Late Weichselian history of relative sea level changes in Iceland during a collapse and subsequent retreat of marine based ice sheet. *Cuadernos De Investigación Geográfica*, *41*, 261–277.

Power, G. (2002). Charrs, glaciations and seasonal ice. *Environmental Biology of Fishes*, *64*, 17–35.

Quinn, T. P. (2018). *The Behavior and Ecology of Pacific Salmon and Trout*, 2nd ed. University of Washington Press.

R Core Team (2020). *R: A Language and Environment for Statistical Computing*. Austria.

Rudolfsen, G., Folstad, I., Sørum, V., & Figenschou, L. (2011). Spawning behaviour of Arctic charr (Salvelinus alpinus): risk of sperm competition and timing of milt release for sneaker and dominant males. *Behaviour*, *148*, 1157–1172. https://doi.org/10.1163/000579511X596615

Sandlund, O. T., Gunnarsson, K., Jónasson, P. M., Jonsson, B., Lindem, T., Magnússon, K. P., Malmquist, H. J., Sigurjónsdóttir, H., Skúlason, S., & Snorrason, S. S. (1992). The Arctic charr *Salvelinus alpinus* in Thingvallavatn. *Oikos*, *64*, 305–351.

Sandlund, O. T., Malmquist, H. J., Jonsson, B., Skúlason, S., Snorrason, S. S., Jónasson, P. M., Gydemo, R., & Lindem, T. (1988). Density, length distribution, and diet of age-0 Arctic charr *Salvelinus alpinus* in the surf zone of Thingvallavatn, Iceland. *Environmental Biology of Fishes*, *23*, 183–195.

Schneider, C., Rasband, W., & Eliceiri, K. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature methods*, *9*, 671–675. https://doi.org/10.1038/nmeth.2089

Sigurjónsdóttir, H., & Gunnarsson, A. (1989). Alternative mating tactics of arctic charr, Salvelinus alpinus, in Thingvallavatn, Iceland. *Environmental Biology of Fishes*, *26*, 159–176.

Skúlason, S., Snorrason, S. S., Noakes, D. L. G., Ferguson, M. M., & Malmquist, H. J. (1989). Segregation in spawning and early life history among polymorphic Arctic charr, *Salvelinus alpinus*, in Thingvallavatn, Iceland. *Journal of Fish Biology*, *35*, 225–232.

Skúlason, S., Snorrason, S. S., Ota, D., & Noakes, D. L. G. (1993). Genetically based differences in foraging behaviour among sympatric morphs of Arctic charr (Pisces: Salmonidae). *Animal Behaviour*, *45*, 1179–1192. https://doi.org/10.1006/anbe.1993.1140

Snorrason, S. S. (2000). Life cycle of the mollusc Lymnaea peregra and its influence on morph formation in Arctic charr, Salvelinus alpinus. *Verhandlungen Des Internationalen Verein Limnologie*, *27*, 1–4.

Snorrason, S. S., Malmquist, H. J., Jonsson, B., Jónasson, P. M., Sandlund, O. T., & Skúlason, S. (1994). Modification in life history characteristics of planktivorous Arctic charr *Salvelinus alpinus* in Thingvallavatn, Iceland. *Verhandlungen Des Internationalen Verein Limnologie*, *25*, 2108–2112.

Snorrason, S. S., Skulason, S., Jonsson, B., Malmquist, H. J., Jonasson, P. M., Sandlund, O. T., & Lindem, T. (1994). Trophic specialization in Arctic charr *Salvelinus alpinus* (Pisces; Salmonidae): morphological divergence and ontogenic niche shifts. *Biological Journal of the Linnean Society*, *52*, 1–18.

Van Dyck, H., Bonte, D., Puls, R., Gotthard, K., & Maes, D. (2015). The lost generation hypothesis: Could climate change drive ectotherms into a developmental trap? *Oikos*, *124*, 54–61.

Warren, D. R., Robinson, J. M., Josephson, D. C., Sheldon, D. R., & Kraft, C. E. (2012). Elevated summer temperatures delay spawning and reduce redd construction for resident brook trout (Salvelinus fontinalis). *Global Change Biology*, *18*, 1804–1811. https://doi.org/10.1111/j.1365-2486.2012.02670.x

SUPPORTING INFORMATION

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How to cite this article: Horta-Lacueva, Q. J.-B., Ólafsdóttir, J. H., Finn, F., Fiskoviča, E., Ponsioen, L., de la Cámara, M., & Kapralova, K. H. (2022). From drones to bones: Assessing the importance of abiotic factors for salmonid spawning behaviour and embryonic development through a multidisciplinary approach. *Ecology of Freshwater Fish*, 00, 1–11. https://doi.org/10.1111/eff.12654

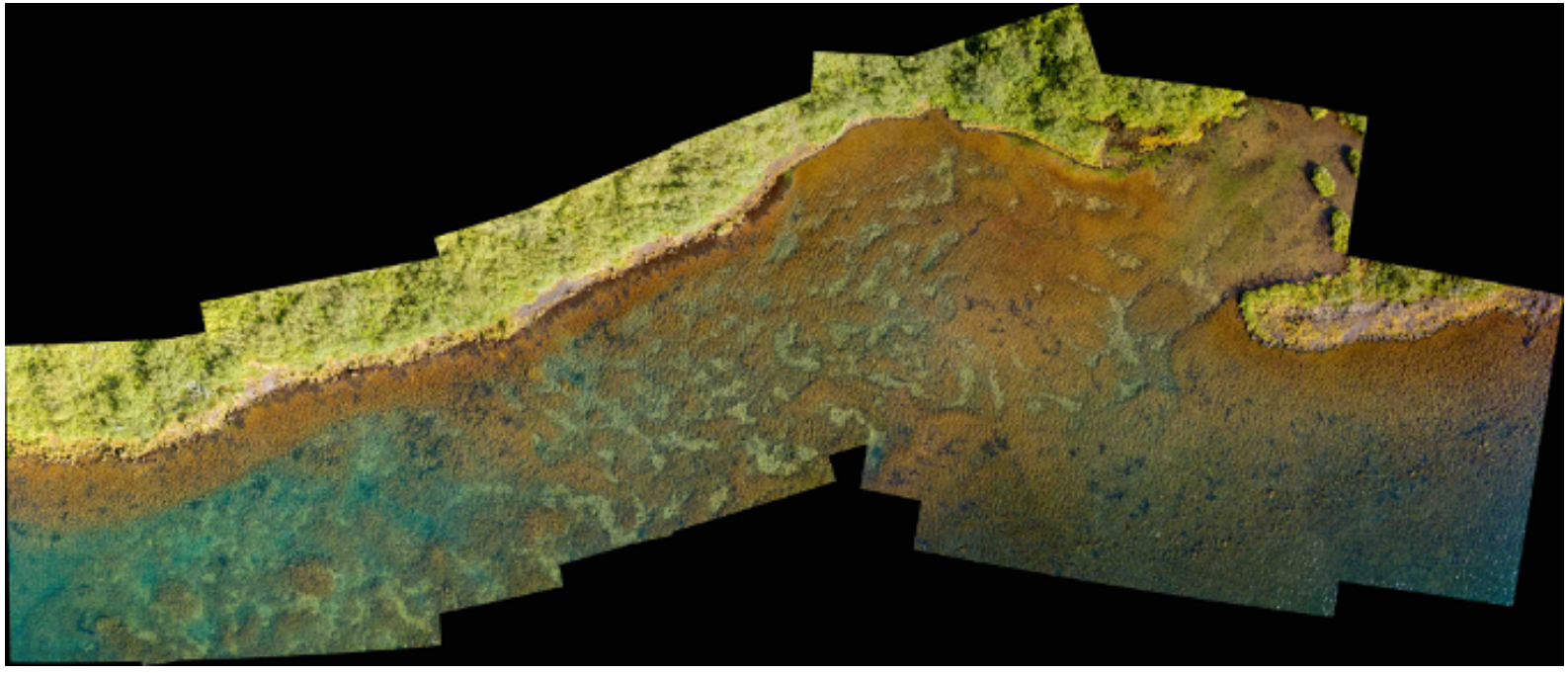

Fig. S1. *Composite image of the LB-charr spawning ground at Ólafsdráttur. Drone footage 50m abot the ground. Fuull size image: https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2Feff.12654&file=eff12654-sup-0001-FigS1.png*

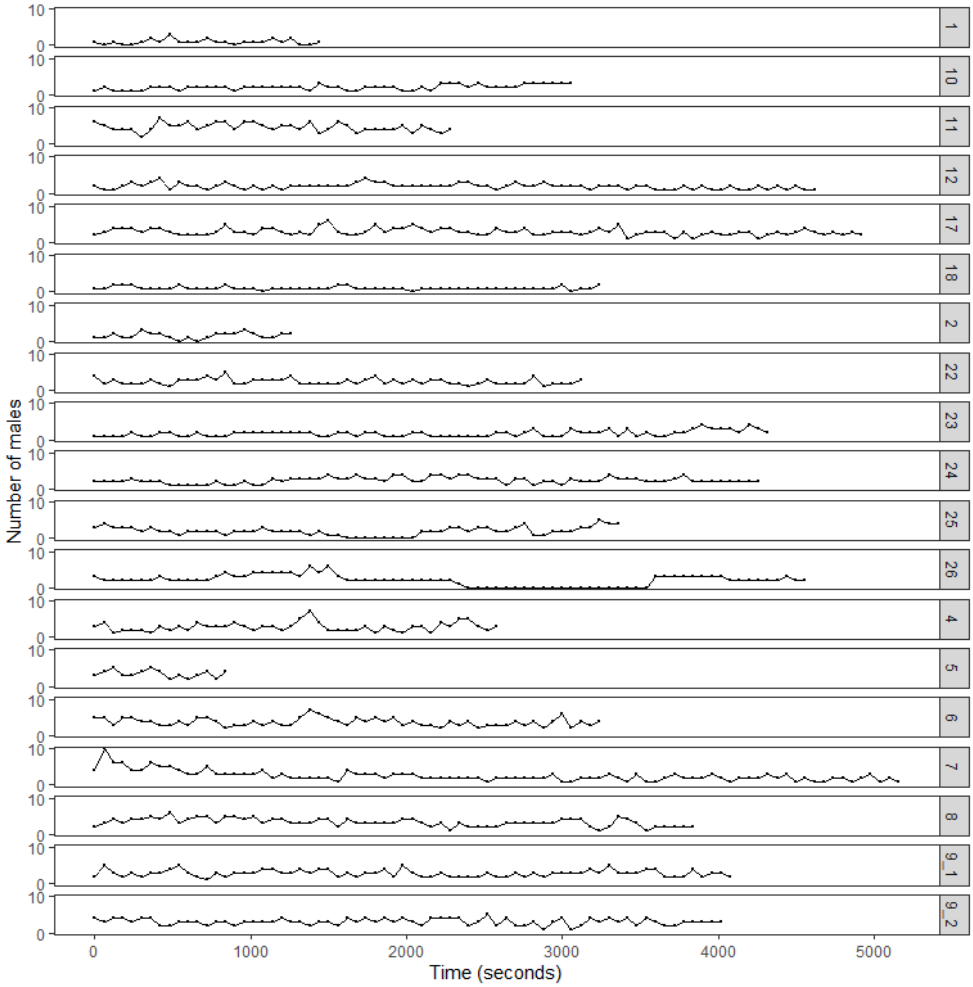

Fig. S2. Number of males counted in the sampled videoframes over time and in each redd.

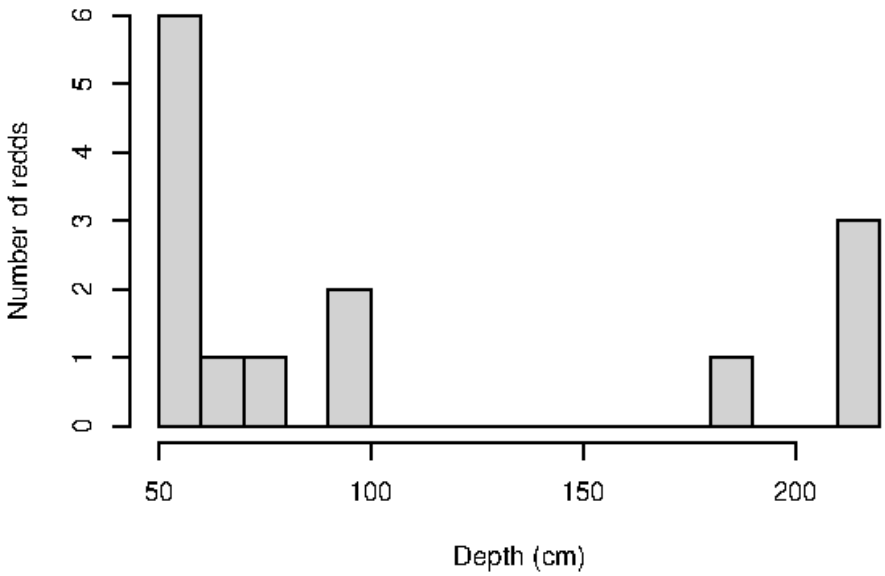

Fig. S3. Distribution of the video recorded redds across depth.