UNIVERSITY OF ICELAND

Ph.D. Dissertation in Biology

The genomic basis of adaptive differentiation between closely related morphs of Arctic charr

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January 2025

FACULTY OF LIFE AND ENVIRONMENTAL SCIENCES

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

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Reykjavik, January 2025

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Bibliographic information:
de la Cámara, M. 2025, *The genomic basis of adaptive differentiation between closely related morphs of Arctic charr*, Ph.D. dissertation, Faculty of Life and Environmental Sciences, University of Iceland, 180 pp.

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ISBN: 978-9935-9807-0-0

Abstract

Exploring the genetic basis of ecological diversification is crucial to understand how diversity is generated and maintained. The overall aim of this thesis is to disentangle the genetic basis behind the ecological differentiation of the Arctic charr in lake Thingvallavatn (Iceland), where this species has diverged genetically and phenotypically into four morphs along the benthic-limnetic ecological axis. Here I focused on studying the genetic underpinnings behind the phenotypic traits involved in this well-characterised case of ecological differentiation: (1) the morphology associated with benthic and limnetic ecologies and (2) the discrete size differentiation. To tackle this, a variety of methodological approaches was used: 1) geometric morphometrics to characterise morphological differentiation across morphs, 2) QTL mapping to map those traits onto the genome, and 3) population genomic approaches to look at the genetic underpinnings behind discrete body size differentiation across morphs. For the geometric morphometrics and the QTL mapping parts of the study, laboratory reared families from the lake were established and for the population genomics part fish were collected from the lake. Throughout this thesis I provide evidence for a genetic basis behind body size and shape in the Thingvallavatn system. QTL mapping revealed that the relative size of the head, maxilla shape and peduncle depth were attributed to single QTL with moderate to high effects, likely complemented with other QTL of small effects. Additionally, genome scans unveiled highly differentiated genomic regions shared between the small and large morph pairs, including a region containing the glypican-6 gene, which is highly conserved in vertebrate evolution, playing a role in cell proliferation and growth. This work significantly contributes to our understanding of ecological diversification and opens avenues for further research in salmonid and other freshwater systems.

To my parents.

Life on earth is more like a verb. It repairs, maintains, re-creates, and outdoes itself.

Lynn Margulis

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This thesis is based on three papers, of which one has been published and two are a manuscript. In the text the papers are referred to with their respective numbers as follows:

Paper I: de la Cámara, M., Ponsioen, L., Horta-Lacueva, Q. J., & Kapralova, K. H. (2023). The dynamic ontogenetic shape patterns of adaptive divergence and sexual dimorphism. Evolutionary Biology, 50(2), 170-180.

Paper II: de la Cámara, M., Jónsson, Z. O. & Kapralova, K.H. (2024). The genomic architecture of benthic-limnetic ecological differentiation.

Paper III: de la Cámara, M., Snorrason, S. S. & Kapralova, K. H. (2024). Genetic processes involved in body size differentiation of the Arctic charr morphs from lake Thingvallavatn.

The author contributions for the three papers are the following:

Paper I: MC conceptualised the study, conducted the analyses and wrote the manuscript. LP and QH collected the data, phenotyped the specimens and critically revised the manuscript. KHK conceived the study, established the crossing design, reared the embryos, collected the data and contributed to the writing of the manuscript.

Paper II: MC conceptualised the study, participated in the sampling of the individuals, conducted phenotyping, laboratory work and genotyping. MC conducted all the analyses and wrote the manuscript. ZOJ made possible the generation of crosses, participated in the construction of the linkage map and revised the manuscript. KHK conceived the study, established the crossing design, reared the embryos, collected the data and contributed to the writing of the manuscript.

Paper III: MC conceptualised the study, participated in the sampling on the individuals, conducted phenotyping, laboratory work and genotyping. MC conducted all the analyses and wrote the manuscript. SSS led the sampling efforts and contributed to the writing of the manuscript. KHK participated in sampling and contributed to the writing of the manuscript.

Associated publications not included in the thesis:

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, 31(3), 596-606.

Abbreviations

AFLP = amplified fragment length polymorphism

CR = chromosomal rearrangement

GBS = genotyping-by-sequencing

GO = Gene Ontology

GWAS = genome wide association studies

IPN = infectious pancreatic necrosis

NGS = next generation sequencing

PR = pelvic reduction

QTL = quantitative trait loci

RAD-seq = Restriction-site associated DNA sequencing

RI = reproductive isolation

RFLP = restriction fragment length polymorphism

SNP = single nucleotide polymorphism

Ss4R = salmonid specific whole genome duplication

Ts3R = teleost specific whole genome duplication

WGD = whole genome duplication

Acknowledgements

I first thank RANNÍS, the doctoral fund of the University of Iceland and the Aðalsteinn Kristjánsson Memorial Fund of the University of Iceland for funding this research.

I‘m immensely grateful to my supervisor Kalina Kapralova for her invaluable support throughout this path. Thanks so much not only for your scientific input, but also for all your help, patience and understanding. I feel extremely lucky to have landed in your lab. I‘m thankful to my committee members, Arnar Pálsson (for your comments, support, energy and excellent admin help), Zophonías Jónsson (for the long coding hours and the beer), Moira Ferguson and Michael Morrisey. I would like to include here a big thanks to Sigurður Snorrason for helping me better understand the ecological side of things.

Thanks to former and present members of the Arctic charr group, who have greatly contributed not only to this thesis, but also to the growth of the human behind. Thanks Quentin, my PhD twin, for being the best buddy. Lieke, my favourite geographer, thanks for the sunny field days, your sense of humour and your empathy. I‘m also grateful to Sébastien for our chats, for your music, and for the paellas in Cádiz; to Guðbjörg, for your support and a resilience that I admire; to Han for your eagerness to help and for your smiles every morning; to Nahal, for being always available to help (not only in the lab), for listening to me, and for bringing your warm smile to Askja every single day; to Marcos for your patience in the lab; also big thanks to Fia, Tryggvi, Kenedy, Sigríður Rut, Dagný, and Kristján. I would also like to thank all the wonderful summer interns who have made excellent contributions to this thesis, and who have taught me how to teach: Elodie Bernier, Fiona Rinaudo, Evan Coulet, Lucille Garcia and Chloé Guilbert. I thank the great professionals at the Natural History Museum of Kópavogur for their work in the field, without which this thesis wouldn‘t have been possible: Finnur Ingimarsson, Haraldur R. Ingvason and Stefán Már Stefánsson.

A huge thanks goes to former and present Askjans, some of which I‘m honoured to call friends: Maite thanks for being such an amazing friend and office buddy; Ayca for understanding me like no-one else does; Jake, thanks for the chats and surfing adventures, and Rob and Kate for opening me the magical and painful world of climbing. Thanks so much Anna for your bright energy and your great advice. Thanks to Dennis, Hannah, Gotje, Ben, Charles, Nico, Noémi, Ian, Ingvild, Mathis, Emily, Bastien, Méline and Elena. Thanks to Fredrik for the lovely trips and plant advice. Big thanks to Marion and Theresa for being the best long-distance PhD buddies. Despite the COVID restrictions I was lucky enough to attend a few in-person courses and conferences, where I made incredible friends. Special thanks Mario for our climbing, surfing and partying moments. Thanks to Jordan, Jero, Julia, Anastasia and Jack.

I can‘t thank enough the absolutely amazing support network that I have in Iceland: first to Tjasa for taking care of me and constantly inspiring me. To my previous flatmates Sophie and Mathilde for having shared a bit of their lives with me and supported me throughout this process. To Bahadir for the adventures we lived together, which you always beautifully capture. Thanks to my lovely aerial hoop gals for taking my mind out of the

office and up in the air: Sara, Zuzana and Urður. Thanks to the Eríal and Kría communities for being so awesome, especially to Anna for becoming such a supportive friend. It‘s impossible to write this without thinking about my wonderful Spanish community in Iceland: thanks for your support, food gatherings, summer houses and music sessions: Óscar, Salma, Mario, Noah and Bryn.

Thanks to my friends in Oslo for always being there: Mohammed and Alia. To Mark for inspiring me, trusting me and getting me into this! I want to thank my friends in Spain, particularly to my biolokitas, Clara, Esther, Ana and Sara. I can‘t believe how much you guys supported me from the distance, thanks for celebrating life and friendship with me during my visits to Madrid. Thanks Joel for listening to me, and for keeping yourself honest, humble and just amazing. Thanks David for always, always being there.

Last but not least, I thank mum and dad for everything, you‘re my pillars and endless sources of strength - thanks for being there unconditionally. To my grandma Paqui for teaching me how to be positive, and to my grandparents Nati, Desi and Paco, who passed away during the completion of this thesis: three stars shine really bright in the nightsky everywhere I go. Thanks to my aunt Nuria for inspiring me so much and for being my confident. Luis, mi amor, thanks for always holding my hand and reminding me what‘s important. Thanks for taking care of me and believing in me, for your beautiful smile and for the unforgettable moments we‘re living together. Daily life with you is by far the most exciting experience I have ever lived.

I would like to dedicate my last sentence to all those young female scientists that may be struggling right now. It‘s a lonely and rough path – remember to reach out for help, your health is more important than anything else. You got this.

1 Introduction

The overall scope of this doctoral dissertation is to disentangle the genetic architecture behind ecological diversification using a non-model organism, the Arctic charr (*Salvelinus alpinus*) in lake Þingvallavatn (Iceland). This is a well-studied system that has historically functioned as a natural laboratory to explore a plethora of evolutionary questions. In this chapter I will frame the topic by reviewing the importance of studying the genetic basis behind ecological specialisation and adaptive radiation to better understand how diversity is generated on a microevolutionary scale. I will later focus on ecological diversification in Northern freshwater systems, especially salmonids and their genomic particularities. I will talk about the striking intraspecific diversity in Arctic charr across its distribution, with a special focus on the benthic-limnetic ecological axis of divergence and discrete size differentiation among morphs (i.e., dwarfism). Lastly, I will detailly describe the Arctic charr system from Thingvallavtn and summarise the genetic research conducted so far.

1.1 The genetics of ecological diversification

Ecological diversification can be defined as the evolution of ecological differences between populations, or subsets of a single population, as a result of divergent selection (Räsänen & Hendry, 2008; Schluter, 2000; Schluter & Conte, 2009) when in presence of novel ecological opportunities (Gavrilets & Vose, 2005; Losos & Mahler, 2010; Rundle & Nosil, 2005). Often, the phenotypic patterns generated by spatially varying selection have a genetic basis, which has been studied in multiple systems across various scales. Numerous studies have documented these on a macroevolutionary level (Bhullar et al., 2012; Hulsey, 2005; Sidor, 2001), among closely related species (Abzhanov et al., 2004; Roberts et al., 2011; Schluter, 2000) and even among populations within a single species (Kimmel et al., 2005; Landry & Bernatchez, 2010; Schluter, 1996; Smith & Skúlason, 1996). Despite the fact that macroevolutionary studies are of crucial importance to understand the evolution of discrete taxonomic units, they bring both conceptual and methodological challenges to the table (Colosimo et al., 2004; Erwin, 2001; Fan & Meyer, 2014; Franchini et al., 2014; Parsons et al., 2016; Seehausen, 2006), but see recent efforts on bridging the gap between macro- and micro- evolution (Pagel et al., 2022; Rolland et al., 2023; Taverne et al., 2021). Microevolutionary studies on the other hand help us better address questions about individual variation, specialisation, adaptation and speciation processes. However, microevolutionary studies also come with challenges, such the difficulty of conducting experiments in natural populations or accurately sampling and measuring the direction of selection and fitness (Merilä et al., 2001; Reznick & Ricklefs, 2009).

To overcome these challenges, some microevolutionary studies benefit from systems which function as evolutionary arenas: these are ideally virtually isolated ecosystems, such as an island or a lake, with relatively simple and well-documented ecological dynamics. These systems are often home to adaptive radiations, a special case of ecological diversification where species or populations diverge in function to their resource utilisation via natural selection in relatively short periods of time. The rapid generation of ecological

and morphological diversity makes adaptive radiations an excellent opportunity to explore ecological diversification at a microevolutionary scale. For adaptive radiations to occur, some form of ecological opportunity is essential (Berner & Salzburger, 2015; Simpson, 1953), such as the colonisation of a novel environment, the radical change of an environment, the appearance of a new resource, or the evolutionary emergence of a key feature or innovation that allows the population to utilise a new resource (Burress & Tan, 2017; Garcia-Porta et al., 2022; Losos & Mahler, 2010). Iconic examples of adaptive radiations include Darwin's finches (Bowman, 1961; Grant, 1999; Grant & Grant, Rosemary, 2007; Lack, 1945), African Rift lake cichlids (Cooper et al., 2010; Fryer, 1972), Hawaiian spiders (Gillespie, 2004), Hawaiian honeycreepers (Amadon, 1950; Freed et al., 1987), Caribbean Anolis lizards (Losos, 2011) or sticklebacks (Bell & Foster, 1994), but see an example of a nonadaptive radiation in (Wellenreuther & Sánchez-Guillén, 2016).

Genomic architecture is defined as the number and magnitude of the effects of loci influencing phenotypic variation (Slate, 2017). The genomic architectures behind most phenotypic traits are complex, as such traits are often affected by multiple genes with different magnitudes of effects. In some cases the direction of effects is conditional, due to various forms of epistasis (Goddard et al., 2016). While the study of the genomic architecture of complex traits is relevant to medicine or agriculture (Buerstmayr et al., 2009; Rothschild et al., 2007; Swamy et al., 2011), it can also provide remarkable insights to understand the genetic basis of naturally occurring variation within species. A textbook example of a relatively simple genomic architecture in an ecological diversification context is the case of *Eda* and *Pitx1,* major loci responsible for the adaptive divergence in the defensive armour across stickleback ecomorphs (Chan et al., 2010; Colosimo et al., 2005; Cresko et al., 2004). Other examples of loci with large effects are the *ALX1* gene, which is strongly associated with beak shape diversity across Darwin's finch species and with the rapid diversification of the medium ground finch (Lamichhaney et al., 2015), or the *optix* gene, responsible for extreme red wing pattern variation across multiple species of *Heliconius* butterflies (Reed et al., 2011). Furthermore, changes in loci located in regulatory regions such as miRNAs can also explain phenotypic variance. For example, *Drosophila melanogaster's* natural variation in the size of the "naked valley" (i.e., a trichome-free area on the femur of the second leg) is mostly explained by changes in mir-92a expression (up to 91% of variation in naked valley size), which regulates the 3'UTR region of the *shavenoid* gene (Arif et al., 2013).

Simple genomic architectures help us understand the genetic mechanisms responsible for ecological diversification, however the genetic basis of ecological diversification is almost never explained by a single phenotypic trait. For instance, animal systems may have complex morphologies, often associated to trophic morphology or other traits related to the access of specific resources, that manifest as changes in multiple anatomical, physiological and even behavioural traits. One such example is the sympatric pupfish population that has diverged into large-jawed scale-eater and a short-jawed molluscivore in San Salvador Island (Bahamas) (Martin et al., 2017). In this study, traits related to trophic polymorphism were assessed by various linear measurements, such as premaxilla and maxilla length, lower jaw length or palatine height, head and body depth, peduncle height or dorsal fin depth. Of this multidimensional phenotype, eight traits were explained by single quantitative trait loci (QTL) with large effects each, distributed across the whole genome (Martin et al., 2017). In other cases, variants on multiple regions correlate with a single trait. For instance, a recent study on African Rift cichlids looking at traits associated with

trophic polymorphism (herbivore *vs* carnivore fish) found multiple QTL of moderate effects across the genome, with pleiotropic effects (Feller & Seehausen, 2022). Another example, this time looking at genetic parallelism in the hypertrophic lips of insectivorous and planktivorous cichlid species, reported a highly polygenic genetic basis, with at least 25 QTL of small, additive effects (Henning et al., 2017)

To add on the already complex picture of the genetic architecture behind ecological specialisation and adaptive radiation, several factors can substantially interact with such evolutionary processes, for example phylogeny (Glor, 2010), demography (Barth et al., 2017; Flohr et al., 2013), development (Hu et al., 2016), behaviour (Tebbich et al., 2010), population structure (Kemppainen et al., 2021) or hybridisation (Arnegard et al., 2014; Seehausen, 2004; Shi et al., 2023). These factors can both facilitate and constrain the pathways to ecological diversification, and they may as well interact with one another. For instance, Kemppainen et al., 2021 found that population structure appears to constrain local adaptation in sticklebacks: the deletion patterns in the *Pitx1* regulatory element were only found in the population in which pelvic reduction (PR) mapped to a specific chromosome, even though large-effect QTL for PR were found in several other populations. These results suggest that the genetic architecture of the PR trait is variable and dependent on the heterogeneity in the spatial distribution of standing genetic variation caused by strong population structuring.

Briefly, I conclude this section by emphasising the importance of exploring ecological specialisation and adaptive radiations to understand how biodiversity is generated and maintained at a microevolutionary scale. The genomic underpinnings behind diverging phenotypes may, exceptionally, be simple, however complex genomic architectures are found to explain the wide variety of complex phenotypic traits observed in nature.

1.2 Ecological diversification in Northern freshwater ecosystems

Certain clades are more prone to experience ecological diversification and adaptive radiation (both in terms of species richness and within-species diversity) for phylogenetic reasons, such as heterogeneity in the rate of diversification among clades or variable clade ages (Burress & Tan, 2017; Wiens, 2011). Furthermore, ecological diversification may occur more often in some types of ecosystems than others, as such ecosystems can provide a release from competition and predation pressures, allowing a clade to diversify into a variety of ecological niches from which they were previously blocked (reviewed by (Stroud & Losos, 2016)). Instances of such ecosystems include islands (Gillespie, 2004; Grant & Grant 2007), and lakes (Sandlund et al., 1992; Seehausen, 2006). Lakes in particular, offer a great opportunity to study the genetics of ecological diversification due to their relatively isolated nature, combined with the absence of geographical barriers that could promote allopatric divergence (Cristescu et al., 2010). This allows to purely focus on the processes of ecological specialisation to different resources in sympatry (i.e., in the same geographical area) (Bolnick & Fitzpatrick, 2007; Dieckmann & Doebeli, 1999).

Admittedly, ancient lakes are valuable systems to study ecological diversification due to their steady limnological ageing processes and low extinction rates. They often provide textbook examples of adaptive radiations, (reviewed by (Cristescu et al., 2010)). There are

however some drawbacks to using such systems to study ecological diversification as they are more likely to have been subject of complex geological and climatic histories, secondary colonisations and other perturbations (Seehausen, 2006). Conversely, extensive new freshwater habitats were formed in the Northern Hemisphere after the last glaciation (hereafter, young lakes) (Snorrason & Skúlason, 2004). Such young lakes have simpler geological histories and less colonisation events and are often viewed as natural laboratories to investigate evolutionary processes such as recent selection and rapid adaptation. Furthermore, young lakes are typically species poor and thus colonisers are able to exploit available (sometimes discrete) resource types with low predation risks, often leading to instances of resource polymorphism (Snorrason & Skúlason, 2004). The high phenotypic and genotypic variability within and among populations and species resulting from such colonisations, followed by rapid diversification events is widespread across the Northen Hemisphere (e.g., sticklebacks, whitefish, trout, salmon and charr (reviewed by (Klemetsen, 2013)).

The body of literature regarding ecological diversification in Northern freshwater ecosystems is mainly dominated by sticklebacks (e.g., (Arnegard et al., 2014; Cresko et al., 2004; Kimmel et al., 2005; Venu et al., 2024). This intense interest is driven by the remarkable cases of parallel ecological adaptation across marine and freshwater environments, as well as along the benthic-limnetic ecological axis. Their capacity to radiate, in combination with the relative simplicity to conduct experiments in captivity, as sticklebacks have small body sizes, short generation times and high fecundity rates (Reid et al., 2021), makes this species an excellent system to investigate ecological diversification and adaptation. Besides sticklebacks, other outstanding cases of ecological divergence in Northern freshwater ecosystems deserve attention. These occurrences are predominantly observed within the *Salmonidae* family, where genetically differentiated sympatric morphs are found across at least five salmonid genera (*Coregonus, Oncorhynchus, Prosopium, Salmo,* and *Salvelinus*) and in populations across their distribution in Asia, Europe, and North America (Salisbury & Ruzzante, 2022). Among these highly variable five genera, salmonid fish diverge across lacustrine and riverine environments, benthic and limnetic feeding habitats and depth gradients (profundal and abyssal) (see details in (Salisbury & Ruzzante, 2022) and references therein). Convergent phenotypic specialisations in these divergent habitats are also widespread across species (Salisbury & Ruzzante, 2022). For instance, differences in head shape and gill raker count are similar among benthic and among limnetic morphs in different populations and species (Fenton et al., 2024; Hudson et al., 2005). Discrete differences in body size represent another important convergent phenotypic specialisation across salmonid species (present in 10 out of the 15 salmonid species that present divergent sympatric morphs *Coregonus artedi, C. clupeaformis, C. lavaretus, O. mykiss, Prosopium spp., Salmo trutta, Salvelinus alpinus, S. S. fontinalis, S. malma* and *S. namaycush*) (Hudson et al., 2005).

The genetic underpinnings behind the ecological diversification in salmonid fish are generally characterised by a lack of parallelism (Brachmann et al., 2022; Jacobs et al., 2020; Landry & Bernatchez, 2010), indicating that different genetic pathways are involved in the phenotypic specialisations mentioned above. This lack of parallelism in salmonids may be driven by genetic capacity, rather than natural selection (Elmer & Meyer, 2011). This idea of a scenario of no apparent selective pressures is further supported by the colonisation of newly formed freshwater systems with ample ecological opportunities and low competition and/or predation. Moreover, higher degrees of discrete ecological

specialisation turn out to be directly correlated with lake size (Doenz et al., 2019; Recknagel et al., 2017).

It is important to note that the genomes of salmonids share unique characteristics that may have contributed to the diversification seen in this clade: whole genome duplication (WGD) events and subsequent chromosomal rearrangements (CR). In addition to the two basal vertebrate WGD (i.e., 1R and 2R) (Dehal & Boore, 2005; Simakov et al., 2020), the ancestor of teleosts went through a third WGD (i.e., teleost-specific 3rd round of WGD, Ts3R, around 350 m.y.a) (Glasauer & Neuhauss, 2014; Hoegg et al., 2004). Subsequently, the *Salmonidae* family underwent another WGD event about 95 m.y.a (i.e., salmonid-specific 4th round of WGD, Ss4R) (Allendorf et al., 1984; Macqueen & Johnston, 2014). The Ss4R consisted of an autotetraploidization event, meaning that the number of chromosomes present in salmonid species doubled. Following the Ss4R, a process of rediploidisation (i.e., a tetraploid genome returns to diploidy (Lien et al., 2016)), has been taking place in salmonid species and populations at different paces (Hale et al., 2021). Rediploidisation involves major CR, including chromosomal fusions, fissions, translocations, inversions and divergence of paralogous genes. Other non-structural changes include the proliferation of transposable elements (TEs) (Christensen et al., 2018). A substantial proportion of the salmonid genome (10–20%) has yet to complete the rediploidization process and still maintains tetraploid genetic characteristics including potential for tetrasomic inheritance (Allendorf et al., 2015). This surplus of genetic material offers an opportunity for mutation, drift or selection to occur (Crow & Wagner, 2006; Feulner & De-Kayne, 2017). Interestingly, cases of gene neofunctionalisation in duplicates unexpectedly exceed those of subfunctionalisation in Atlantic salmon (Lien et al., 2016). The variation generated by both the WGD events and the rediplodisation processes can greatly impact evolutionary change, decreasing extinction risk and promoting adaptation (Baduel et al., 2018; Crow & Wagner, 2006).

Besides the work focussing on the salmonid's special genomic characteristics, researchers have generated a striking amount of available, high-quality genetic resources, primarily due to their economic and cultural significance as both farmed fish and wild angling stocks. Technological advances including affordable sequencing methods and computational tools only boosted the enormous research investment seen these days, often substantially supported by the aquaculture industry (Houston & Macqueen, 2019). Breeding programs of Atlantic salmon were first developed in Norway early in the 1960s (Houston & Macqueen, 2019; Yáñez et al., 2014), and since then, the availability of resources only increased with the interest in selecting phenotypic traits for breeding purposes. An inflexion point in salmonid research was the release of an Atlantic salmon proto-linkage-map, developed in 1997, which contained only a few markers (Slettan et al., 1997). The purpose of this map was to detect loci related to variation in phenotypic traits of interest, mostly size and condition factor. A more expanded linkage map of the rainbow trout was released only a year later, counting with around 500 markers. In the first decade of the 2000s, linkage maps of multiple salmonid species were published. These were mainly constructed using microsatellite markers , AFLPs and/or allozyme markers (e.g. (Gharbi et al., 2006; Gilbey et al., 2004; Guyomard et al., 2012; Küttner et al., 2011; McClelland & Naish, 2008; Moen et al., 2008; Rexroad et al., 2008; Sakamoto et al., 2000; Woram et al., 2003). With the development of new methods that allowed for a better coverage of the genome and relative randomisation of markers, such as Restriction-site associated DNA sequencing (RADseq, (Baird et al., 2008)) and genotyping-by-sequencing

(GBS, (Elshire et al., 2011)), linkage maps greatly increased their marker density (e.g., (Gonen et al., 2015; Hale et al., 2017; Kodama et al., 2014; Leitwein et al., 2017; McKinney et al., 2016). More recently, SNP arrays have been used to generate linkage maps and improve the assemblies of the available reference genomes (Christensen et al., 2018; Tsai et al., 2016) and others, publicly available in (https://salmobase.org/). Again, the most studied phenotypic traits are body weight and condition factor (e.g., (Gutierrez et al., 2012; Reid et al., 2005; Sauvage et al., 2012), but also other life history traits, such as size and age at maturity (Barson et al., 2015) or susceptibility to diseases such as the infectious pancreatic necrosis (IPN) (Houston et al., 2010). These traits are evidently important in aquaculture production and conservation of wild stocks, but they can also have broader implications, as many of them are, or are related to, phenotypes involved in ecological specialisation.

1.3 A highly polymorphic species: the Arctic charr

The genus *Salvelinus* has a Northern circumpolar distribution, inhabiting freshwater bodies, although some species and populations also migrate to the ocean, retaining the classical salmonid trait of anadromy. *Savelinus* is a genus characterised by significant levels of inter- and intra-species polymorphisms, seen in both allopatry and sympatry (Klemetsen, 2013). Highly polymorphic charr populations are widely present across the species distribution, for instance in Russia (Markevich et al., 2018), Norway (Grenier et al., 2021; Østbye et al., 2020), Scotland (Fenton et al., 2024), Canada (Bertrand et al., 2008; Salisbury et al., 2018), Greenland (Doenz et al., 2019) and Iceland (Woods et al., 2012), among other Holarctic locations. One of the most drastic cases of discrete morphological *Salvelinus* polymorphism occurs in lake Kronotskoe (Kamchatka, Russia), a large, deep lake that homes seven morphs of Dolly Varden charr (*S. malma*) that differ in size, colouration, spawning habits, morphology and diet (Markevich et al., 2018). Another example is the Arctic charr (*S. alpinus*) diversification in at least two lakes in Greenland, where six charr morphotypes can be found (Doenz et al., 2019) (***Fig. 1***).

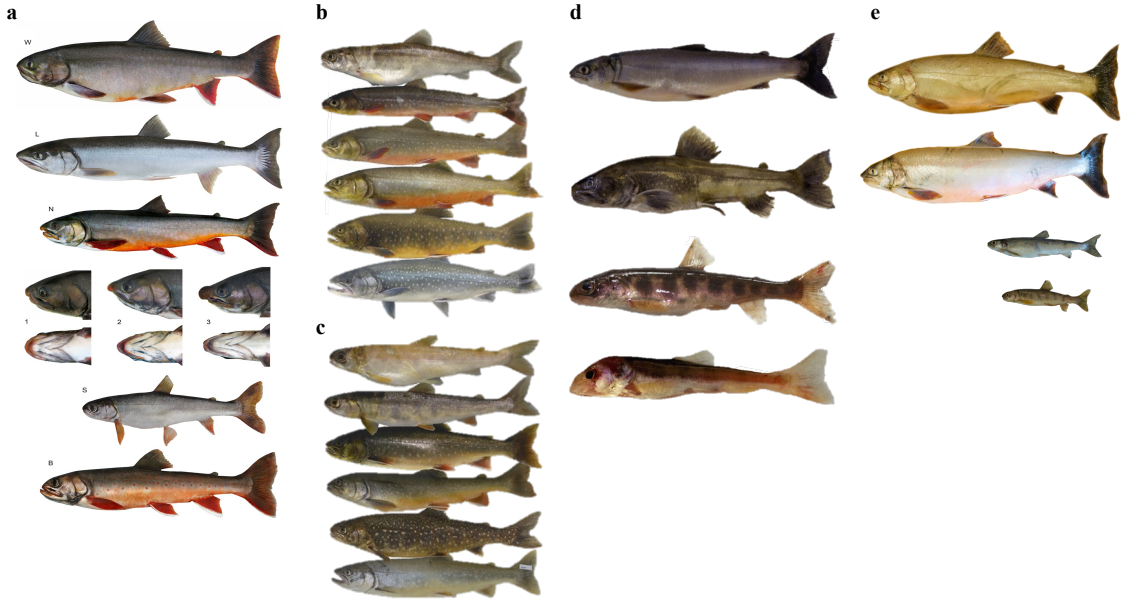

Figure 1. *Examples of sympatric ecological diversification in charr (*Salvelinus spp.*),* ***not*** *to scale. (a) The seven morphs of Dolly Varden charr (*S. malma*) from lake Kronotskoe (Kamchatka, Russia), (b) the six morphs of Arctic charr (*S. alpinus*) from lakes Tasersuaq and (c) Saqqaata Tasia (Greenland), (d) the four Arctic charr morphs from lake Tinnsjøen (Norway) and (e) the four Arctic charr morphs from lake Þingvallavatn (Iceland). Pictures modified from (Markevich et al., 2018)(a), (Doenz et al., 2019)(b,c), (Østbye et al., 2020)(d), (de la Cámara et al., 2024)(e).*

The most widespread cases of phenotypic polymorphisms are observed in the Arctic charr (*S. alpinus*) in at least 40 lakes across its Holarctic distribution (Jonsson & Jonsson, 2001; Wilson et al., 2004). Historically, the Arctic charr became isolated into multiple glacial refugia during the Pleistocene glaciation (Bernatchez & Wilson, 1998; Hewitt, 2000), resulting in five lineages (i.e., Acadian, Atlantic, Arctic, Bering and Siberian) that developed in allopatry and are at present genetically differentiated from one another (Brunner et al., 2001, 2001; Moore et al., 2015). After the last glacial retreat, the Arctic charr re-colonised multiple lacustrine systems, in some cases still retaining its anadromy, which seems to have a genetic component (Salisbury et al., 2022). Despite the fact that the Arctic charr recolonisation of Northern freshwater systems has allowed for secondary contact among some of the five lineages (Jacobsen et al., 2022; Salisbury et al., 2019), other populations have remained isolated and developed polymorphisms in sympatry, often via resource polymorphism (Jacobsen et al., 2022; Snorrason & Skúlason, 2004), representing excellent examples of ecological diversification. The most common ecological axis of divergence resulting in population polymorphism in Arctic charr is the benthic-limnetic (i.e., bottom feeders *vs* pelagic feeders), which is common in the ecological diversification of other teleosts (Härer et al., 2021; Kusche et al., 2014; Malmquist et al., 1992). Interestingly, the ecological divergence along the benthic-limnetic ecological axis persists across the Arctic charr distribution, despite populations diverging through different evolutionary histories, from a single colonisation followed by sympatric differentiation (Brachmann et al., 2021) to allopatric divergence and secondary contact (Garduño-Paz et al., 2012; Jacobs et al., 2020).

1.3.1 The benthic–limnetic ecological axis of divergence

The ecological specialisation in benthic and limnetic environments seems to be a key driver of morphological diversity in Arctic charr. Morphological characteristics associated with utilising benthic habitats (i.e. from the bottom of the water body) are present mostly in the trophic apparatus, for instance benthic fish tend to have subterminal mouths (i.e., the lower jaw is shorter than the upper jaw) and rounder snouts. Other benthic-like morphological traits comprise deep bodies, large heads in relation of the body, deep pectoral fins, darker colourations and smaller eyes. On the other hand, limnetic fish obtain resources from the open-water column. To better catch moving or floating preys, limnetic fish have pointy, terminal or supraterminal mouths (lower jaw and upper jaw have the same length, or the lower jaw is longer than the upper jaw, respectively). Limnetic fish are also characterised by slender bodies, small heads, light colourations and larger eyes (Brachmann et al., 2021; Jonsson & Jonsson, 2001; Kristjánsson et al., 2018; Salisbury & Ruzzante, 2022; Sandlund et al., 1992). Despite these general differences between bottom and water column feeding Arctic charr, considerable shape variation exists within benthic and limnetic morphotypes. Such shape variation often depends on the population's evolutionary history, available ecological opportunities, and/or anthropogenic effects (Grenier et al., 2021; Østbye et al., 2020; Parsons et al., 2010) (ref mined lake). For instance, deeper lakes may offer a wider range of ecological opportunities along the water column, leading to the establishment of different benthic morphs. For example, the Arctic charr in lake Tinnsjøen (Norway) (460m deep) has diverged into four morphs along a depth gradient. These are one limnetic, planktivorous morph which utilises resources in the pelagic zone, and three littoral-benthic morphs: a dwarf, a piscivorous and an abyssal charr that utilise different food resources from the littoral bottom to the deepest areas of the lake. The four morphs are morphologically and genetically differentiated, with variable barriers to reproductive isolation among each other (Østbye et al., 2020).

The genetic component behind benthic and limnetic Arctic charr morphs has been reported in multiple locations using a myriad of approaches (e.g. (Ahi et al., 2015; Brachmann et al., 2022; Guðbrandsson et al., 2019; Kess et al., 2021; Küttner et al., 2014)), although the effects of phenotypic plasticity can play an important role in morphological variation between morphs (Adams & Huntingford, 2004; Grünbaum et al., 2007; Kristjánsson et al., 2018; Lien et al., 2016; Parsons et al., 2010; Þórhallsson 2022). Four main approaches have been used in the charr literature to study the genetic basis behind the phenotypic differences among morphs, two of them use individuals reared in captivity: (1) the analysis of shape variation in common-garden conditions, and (2) QTL mapping, while the other two use sampling from the wild populations: (3) population genetics and genome scans and (4) genome-wide association studies (GWAS). I will now briefly summarise the main findings in the Arctic charr benthic-limnetic diversification by approach.

The genetic component of traits associated to benthic-limnetic lifestyles of charr has been explored by rearing benthic and limnetic fish in captivity and exploring the shape variation in the progeny (approach 1). This is an indirect, yet informative way to focus on shape variation by reducing the effects of phenotypic plasticity (though not controlling transgenerational or epigenetic induced plasticity), which appear to be non-negligible in multiple sympatric pairs of Arctic charr (Adams & Huntingford, 2004; Grünbaum et al., 2007; Parsons et al., 2010; Þórhallsson, 2022). By using captivity common garden it has

been shown that in Arctic charr there is a genetic basis to traits traditionally associated to benthic and limnetic feeding strategies (e.g. (Horta-Lacueva et al., 2021; Kapralova et al., 2015; Klemetsen et al., 2006; Parsons et al., 2010; Ponsioen, 2020; Skúlason et al., 1989).

QTL mapping makes use of families reared in captivity to construct linkage maps and subsequently map the phenotypic traits of interest (approach 2). This approach takes advantage of linkage disequilibrium (LD) between alleles at a locus that affects a quantitative trait and markers in the physical vicinity of that locus. Due to the interest in genetically improving salmonids for commercial purposes, a plethora of QTL mapping studies have been conducted for the salmonid family, including the Arctic charr (Gutierrez et al., 2012; Küttner et al., 2011; Ouellet-Fagg, 2023; D. P. Reid et al., 2005; Vasemägi et al., 2010). Although these studies often focus on traits of importance to aquaculture practices, there are instances where traits related to ecological specialisation were mapped onto the genome. The most recent effort consisted of exploring the developmental architecture of morphological traits focusing on the effects of ontogeny (age) and environment (diet, related to trophic polymorphism and hence benthic-limnetic adaptations) (Ouellet-Fagg, 2023). In this study a new linkage map of Arctic charr was constructed by incorporating both Icelandic and North American populations and combining microsatellites, sex-determining genes and SNPs. Furthermore, body shape and body size were reported to have highly polygenic architectures, with few cases of QTL co-localisation. One of the QTL co-localisations was found to be on linkage group AC11, and appeared to explain variation in different of traits (i.e., jaw length, eye diameter, body depth, caudal fin depth and body size). Previously, a less dense Icelandic Arctic charr linkage map using only inbred lines was employed to perform QTL mapping on body weight, condition factor, and age of sexual maturation, traits significant to both aquaculture practices and ecological studies (Küttner et al., 2011). In both studies, the genetic basis of the morphological traits was found to be polygenic, with QTL accounting for moderate to high effects.

Population genetics studies (approach 3) focus on the unbiased assessment of genetic differentiation between benthic and limnetic morphotypes in natural populations, mainly neutral markers, but can also identify those shaped by positive selection. Studies on multiple Arctic charr systems have found more or less discrete genetic clusters and certain levels of introgression among morphs in sympatry (Adams et al., 2008; Brachmann et al., 2022; Fenton et al., 2024; Guðbrandsson et al., 2019; Hindar et al., 1986; Küttner et al., 2014). Within this population genetics context, genome scans use descriptive statistics of allele frequencies and genetic variations to find differentiated regions of the genome. Population genomics studies in Arctic charr have reported a general pattern of genome-wide divergence, with high F_{ST} markers on possibly all linkage groups, rather than genetic islands of differentiation on few linkage groups. For instance, (Guðbrandsson et al., 2019) argues that modest, genome-wide differentiation between benthic and limnetic morphs from lake Þingvallavatn (Iceland) implies that ecological specialisation was likely coupled with the establishment of prezygotic barriers (such as spatial and temporal separation during spawning or behavioural differences) that have kept the sympatric benthic and limnetic populations reproductively isolated for some time. However, a few islands of differentiation have also been found when trying to disentangle the genomic basis behind benthic and limnetic sympatric populations (Guðbrandsson et al., 2019).

While the population genetics approach focuses on genetic variation between groups, after specimen categorisation based on multiple factors (i.e., morphological, ecological), GWAS (approach 4) utilises the variation within groups. Hence GWAS represents a more targeted approach to identify the genetic component related to specific phenotypic variation from the natural population. Such studies are looking at statistical associations between morphological variation related to benthic and limnetic specialisations and genomic variation (Gienapp et al., 2017; Santure & Garant, 2018). The main challenge of this method is integrating the association signal with phylogenetic relatedness, although this has been addressed in several systems (Eu-ahsunthornwattana et al., 2014). In Arctic charr GWAS have been used to both report regions of the genome enriched with these associations and list candidate genes, and also to study the extent of genetic parallelism behind benthic-limnetic phenotypes across locations and charr lineages (Fenton et al., 2024; Jacobs et al., 2020). These studies found that there is rather limited allelic and genetic parallelism behind benthic-limnetic Arctic charr phenotypes, and that the extent of this genetic parallelism may depend on the geographical context and demographic history of the populations, especially when comparing to other species and systems such as sticklebacks (e.g., with GWAS., (Härer et al., 2021)).

1.3.2 Discrete size differentiation

Discrete size differentiation is widely observed in multiple sympatric pairs of Arctic charr populations across it distribution (Salisbury & Ruzzante, 2022). Miniaturisation or dwarfism is the drastic reduction in body size in relation to an ancestral population, and seems to allow the derived dwarf populations to occupy a different niche and access different resource types. Furthermore, this evolutionary strategy is often interacting with other differences related to niche exploitation. For instance, dwarf fish are also associated with benthic or limnetic ecologies (Malmquist et al., 1992; Skúlason et al., 1989), profundal lifestyles (Kess et al., 2021; Østbye et al., 2020; Præbel et al., 2016), insectivorous diets (Berg et al., 2010) or other ecological habits (Kristjánsson et al., 2012).

As mentioned above, divergence in body size in found in many polymorphic Arctic char populations and the evolutionary strategies for becoming large or small are diverse. One of the most accepted explanations for the repeated pattern of dwarfism across Arctic charr populations has to do with paedomorphism, which is a type of heterochrony (an evolutionary change in rates and timing of developmental processes (Klingenberg, 1998)). In a context of ecological specialisation, evolution via paedomorphism generates morphological divergence by retaining juvenile characteristics (e.g., (Bhullar et al., 2012)), such as small body size, juvenile colouration, occupation of nursery habitats or preferences for a juvenile diet (Kristjánsson et al., 2018; Parsons et al., 2011; Pichugin et al., 2023; Sigursteinsdóttir & Kristjánsson, 2005; Skoglund et al., 2015; Skúlason et al., 1989). From a physiological point of view, large and small Arctic charr morphs seem to differ in muscle fibre recruitment (Johnston et al., 2004). The main result from Johnston et al study was that the recruitment of fast muscle fibres ceases at a defined body length, that seems to be characteristic of each morph, after which growth is mostly explained by hypertrophy of fibres already present earlier in development. The same study supports the idea that fast fibre muscle recruitment likely has a genetic basis.

The same group also studied the physiology of growth, to explore the genetic basis of the parallelly evolved dwarf charr. This was investigated by manipulating dietary variables in

five dwarf and two generalist charr populations in a common garden experiment, and looking at variation in the expression of genes involved in the m-TOR pathway (involved in cellular growth) in musculoskeletal cells (Macqueen et al., 2011). This study detected three candidate genes (i.e., *mTOR*, *4E-BP-1* and *IGFBP4*) that showed significant differential expression patterns between the dwarf and the generalist populations.

1.4 The Arctic charr in Þingvallavatn

Iceland is an excellent location for exploring resource polymorphism in Arctic charr. The main reason is because the Arctic charr systems in Icelandic freshwater bodies tick all the boxes mentioned in section 1.1.: young, practically isolated, absence of secondary colonisations and their ecologies are often well described in the literature. Furthermore, both the benthic-limnetic and the small-large axes of divergence are repeated in multiple lakes (reviewed by (Salisbury & Ruzzante, 2022). Although extant, the genetic and molecular mechanisms behind a potentially parallel ecological divergence seem to be rare (Brachmann et al., 2022; Jacobs et al., 2020). In this section I focus on the Arctic charr populations inhabiting one particular Icelandic lake, Þingvallavatn, located in the South-east of the island.

Lake Þingvallavatn (Iceland, **(*Fig. 2a*)**) is home to one of the most iconic examples of rapid ecological diversification in sympatry in the field of evolutionary biology, with four Arctic charr morphs that have diverged along the benthic-limnetic ecological axis and present dramatic size differences within the benthic and limnetic types **(*Fig. 2b*)**. Þingvallavatn is the largest lake in Iceland, with an area of 83km^2 and maximum depth of 114m. It is a meso- to oligotrophic lake located in a neo-volcanic zone in Þingvellir National Park, in the South-West of Iceland **(*Fig. 2a*)**(Sandlund et al., 1992). Multiple discrete habitats exist within the lake which offer different resources (i.e., ecological opportunities) available for the fish to utilise. Briefly, there is a large pelagic zone with a high phytoplankton production and a benthic zone, the later subdivided in three main areas: (1) the stony littoral zone (0-10m deep), characterised by a hard bottom with scattered stony substrate, porous lava stones and cervices, allowing for a highly diverse habitat for the zoobenthos, (2) the Nitella zone (10-25m deep), a densely vegetated area of the green algae *Nitella opaca*, with abundance of three-spined sticklebacks, and (3) the profundal zone (> 25m deep), formed by a soft bottom with diatomic gyttja substrate (Malmquist et al., 1992; Sandlund et al., 1987, 1992; Snorrason et al., 1994). The fish community is mostly comprised by Arctic charr, but three-spined sticklebacks (*Gasterosteus aculeatus*) and brown trout (*Salmo trutta*) can also be found in Þingvallavatn. Notably, the population numbers of the trout are alarmingly increasing in the lake and may even overtake the Arctic charr population, as observed in a monitoring survey in 2019 (Snorrason et al., *in prep*). Such shifts in the charr and trout numbers may either (i) reflect a natural rebound, as the trout population in the lake collapsed after the damming of Efra Sog in the middle of the last century, or (ii) be the result of the increasing temperature of Þingvallavatn in the last 30 years (Malmquist et al., 2020)(in Icelandic) which may have affected the Arctic charr's reproductive rate, since its development is highly sensitive to temperature increases and fluctuations (Jeuthe et al., 2016; Jonsson & Jonsson, 2014; Muhlfeld et al., 2024).

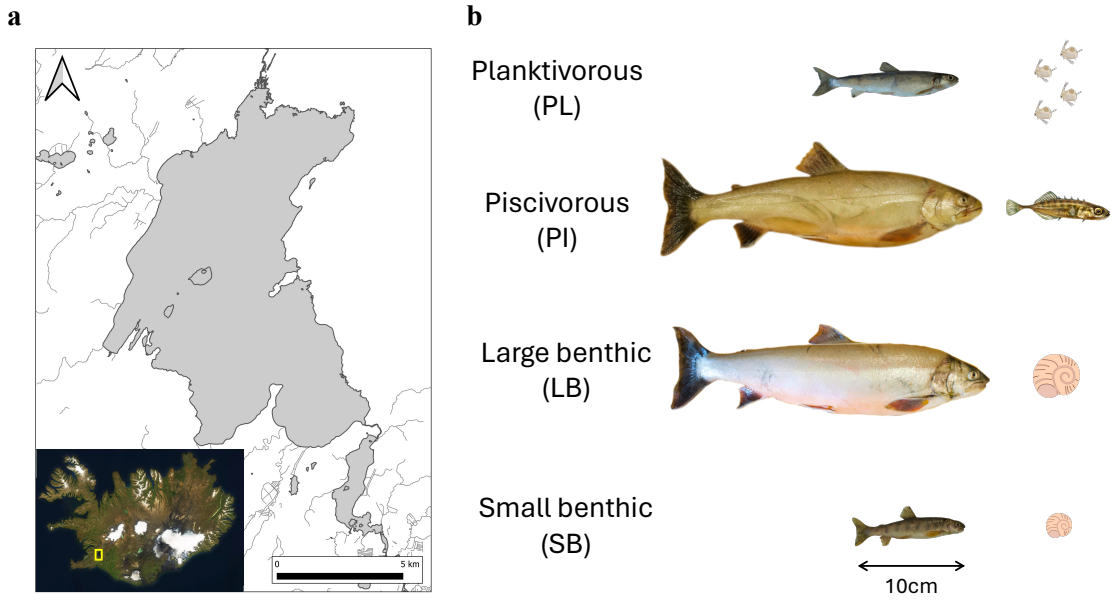

Figure 2. *(a) Map of Thingvallavtn and its location in Iceland (inset), location of the lake depicted with the yellow square. (b) The four Arctic charr morphs inhabiting Þingvallavatn. To the right, their preferred (i.e., Daphnia as an example of zooplankton for the PL charr, three-spined stickleback, preferred diet of the predator PI charr and snails for the LB and SB charr. The fish but not food resources are to scale.*

Þingvallavatn was colonised after the last glacial retreat (10-12000 y.a.) by an anadromous charr ancestor during a relatively short colonisation window, that closed when unscalable waterfalls formed in the outflowing river (Ingólfsson et al., 1995). The colonising fish likely resembled the present-day anadromous charr, common in Icelandic rivers, in being a generalist feeder and maturing at a large size (Bengtsson et al., 2023). Since then, the Arctic charr in the lake has diverged into four morphs, two benthic, the small (SB) and the large benthic (LB), and two limnetic, a small planktivorous (PL) and a large piscivorous (PI). Coalescence simulations have pointed towards a rapid divergence of PL-, SB- and LB-charr probably involving microallopatry followed by secondary contact and sympatry (Brachmann et al., 2022; Kapralova et al., 2011). At present these morphs are thought to form three clear genetic clusters (Brachmann et al., 2022; Guðbrandsson et al., 2019). Our knowledge on the genetic status of the PI morph is yet limited: some hypotheses point towards the PL and the PI charr forming the same population, with the detail that the PI charr likely learned how to prey on sticklebacks (Malmquist et al., 1992; Snorrason et al., 1994). To some extent this has been corroborated by recent genetic studies, but at the same time these data show clear signs of genetic connectivity between PI-charr and LB-charr (Guðbrandsson et al., 2019; Skúlason et al., 1989).

The four Arctic charr morphs are visually distinguishable as adults, diverging from each other in a variety of traits, including size, morphology and colouration, also habitat use, diet, behaviour, parasitic load, time to sexual maturation and spawning times (***Table 1***) (Malmquist et al., 1992; Sandlund et al., 1992). During their first year, juveniles of all morphs share their nursery habitat in the littoral zone, feeding mostly in chironomids. While the SB charr retains the charr juvenile traits (morphology, colouration, parr marks

and diet preferences etc) and occupies the stony littoral zone throughout their life, the three others undergo an ontogenetic shift after their first year of life. This characteristic ontogenetic pattern of the SB charr has been previously described as a case of paedomorphism (Eiríksson et al., 1999), possibly representing how selection affected a developmental mechanism to promoting ecological specialisation. It is important to note that craniofacial morphology during early developmental stages is highly dependent on other factors such as offspring size and female egg size, highlighting the importance of maternal effects across development (Beck et al., 2020).

As adults, the two benthic morphs (SB and LB charr) have a typical benthic morphology while greatly differing in size. They both feed on the zoobenthos, however their diets are slightly different: the SB charr feeds almost exclusively on *Lymnea peregra* snails from the interstitial spaces of the lava bottom, while the LB charr has a more varied diet in the benthic and epibenthic area of the stony littoral. The two limnetic morphs (PL and PI charr) have a classic limnetic morphology and also differ dramatically in size, generating a replicate of the benthic-limnetic axis within each size category. The two limnetic morphs start feeding on zooplankton after the first ontogenetic shift. While the PL charr continue feeding on zooplankton on the open water column for the rest of their life, the PI charr grow larger and undergo a second ontogenetic shift towards piscivory, feeding mostly on sticklebacks in their adult life, generally in the *Nitella* zone where they are abundant (Eiríksson et al., 1999; Guðbrandsson et al., 2019; Skúlason et al., 1989) (***Table 1***). The PI charr thus retains a lifestyle most resembling the anadromous charr, and is considered ancestral, while the remaining three morphs are derived forms.

Table 1. *Summary of the main phenotypic differences among the four Arctic charr morphs from Þingvallavatn.*

	Small benthic (SB)	**Large benthic (LB)**	**Planktivorous (PL)**	**Piscivorous (PI)**
Feeding habitat	Benthic. Interstitial spaces of the lava bottom.	Benthic and epibenthic in the stony littoral zone.	Pelagic.	Pelagic and epibenthic in the *Nitella* zone.
Size (mean fork length)	Small (13 cm).	Large (35cm).	Small (20cm).	Large (36cm).
Morphology	Subterminal jaws, rounder snouts, large and deep heads, deep bodies.		Terminal jaws, pointy snouts, small and narrow heads, slender bodies.	
Colouration (outside the spawning period)	Dark brown with parr marks.	Dark brown (but lighter than SB).	Silver.	Light brown.
Main diet (> 1 year)	Mostly snails (*Lymnaea peregra*).	Varied diet of macroinvertebrates, including *L. peregra.*	Crustacean zooplankton and chironomid pupae.	Three-spined sticklebacks (*Gasterosteus aculeatus*).
Age to sexual maturation	2 years (males) and 4 years (females)	8 years	4years (males) and 5 years (females)	6 years
Spawning times	August – November	July – August	September – November	September – November
***Diphyllobothrium* load**	Very low	Very low	High	High

The four Arctic charr morphs also differ in their parasitic loads (***Table 1***). A total of seven parasites have been found to infect the charr population from Þingvallvatn, although their presence and intensity varies across morphs, here exemplified by the cestode *Diphyllobothrium spp*. The two limnetic morphs had the highest prevalences, intensities and densities of *Diphyllobothrium,* while the two benthic morphs are only slightly infected. This differential parasitic load is explained by the differences in trophic preferences between the limnetic and benthic morphs: both the PL and the PI morph eat copepods (mostly *Cyclops spp.*, an intermediate host of *Diphyllobothrium*), either directly from the zooplanktonic pool or indirectly from infected sticklebacks (discussed in (Sandlund et al., 1992)). Interestingly, the differential parasitic exposure across morphs has been shown to explain early natural divergent selection in the lake (Franklin et al., 2018).

Regarding their life history traits, the two large charr morphs have a longer period to sexual maturation compared to the two small morphs. While all four morphs spawn in the littoral zone, our knowledge on the specific spawning locations is limited (***Table 1***). For instance, the complex reproductive behaviour of the LB charr (Sigurjónsdóttir & Gunnarsson, 1989) has led to the exploration of redd selection in its main known spawning site, the Ólafsdráttur area, located in the northeast part of the lake (Horta-Lacueva et al., 2022). In this study, the LB charr was found to compete for areas in proximity to glacial outlets, where the temperature is colder and more stable, which likely has an impact on the viability of the embryos (Horta-Lacueva et al., 2022). The second particularity about the spawning behaviour in the LB charr is their timing: they spawn unusually early in comparison with the three other morphs (Skúlason et al., 1989), which likely relates to the early seasonal burst of their main prey, the adult snail *Radix peregra* (Skúlason et al., 1989; Snorrason, 2000). This temporal separation of the LB charr spawning season is thought to represent a pre-zygotic barrier between this morph and the other three, and thus increase reproductive isolation. Nevertheless, instances of hybridisation exist between the LB charr and both the SB and the PI charr (Brachmann et al., 2021; Guðbrandsson et al., 2019).

1.4.1 Genetic differentiation among the four Arctic charr morphs

The first observations of discrete morphological differentiation among the Arctic charr morphs from Þingvallavatn were reported in 1840 by the Icelandic naturalist and poet Jónas Hallgrímsson (reviewed by (Jónasson, 1992)). Around sixty years later, the first taxonomic identification of the three fish species present in the lake was reported, together with the first description of four Arctic charr “types”. Already in the decade of the 1980s, the Arctic charr started to receive attention regarding its process of ecological differentiation in sympatry across its distribution (Nyman et al., 1981). In Þingvallavatn, the ecological characteristics of the four morphs were further explored in terms of diet differentiation, morphology and habitat, and published in national (Malmquist et al., 1985) and international journals (Malmquist et al., 1992; Sandlund et al., 1987).

The first study investigating the genetic differentiation among the Arctic charr morphs from Þingvallavatn was published in 1987 (Magnusson & Ferguson, 1987). In this study, 36 allozymes were analysed, revealing little to no genetic differentiation among morphs, except for the SB charr, which was significantly different from the rest. Subsequent studies based on rearing experiments in common garden conditions (with no genetic data) reported evidence for a genetic basis of shape (Skúlason et al., 1989) and of life history traits (i.e.,

growth trajectory and age to sexual maturation) (Skúlason et al., 1996). In the same year, (Volpe & Ferguson, 1996) used different types of makers (i.e., the mitochondrial control region, RFLPs and minisatellites) to reveal a modest genetic differentiation among charr morphs, except for the PI charr. With the advent of population genetics studies, (Wilson et al., 2004) reported a heterozygote deficit within the Þingvallavatn charr population, an indicative of population structuring. On a molecular level *Pax7* (i.e., paired box protein 7), a gene involved in cranio-facial, skeletal muscle and central nervous system development was found to differ between between the LB and SB charr (Sibthorpe et al., 2007).

Later, the scientific interest into population genetics approaches instigated more exhaustive population genetics analyses among the charr morphs from Þingvallavatn, confirming that SB, LB and PL charr constituted different genetic clusters (note that the PI charr was not sampled in the study) (Kapralova et al., 2011). Notably, the authors also conducted backwards simulations to reconstruct the evolutionary histories of the SB and PL charr, and the best supported model indicated that these two morphs underwent a short micro-allopatry period followed by secondary contact and have lived in sympatry since. This model was later confirmed by (Brachmann et al., 2022), who added the LB morph to the coalescence models. In the same year (Küttner et al., 2011) generated the first linkage map from artificial Arctic charr lines derived from the Icelandic charr populations (including Þingvallavatn), and mapped body weight, condition factor and age of sexual maturation. QTL with genome-wide significance had modest to moderate effects and were detected sparsely across the genome with limited evidence for colocalization. Although this study did not focus on genetic differences between ecomorphs, the linkage map represented a valuable genetic resource for the Arctic charr community. The chosen phenotypic traits, clearly important for aquaculture, also play a key role in ecological specialisation.

With transcriptomic methods becoming more readily available, it became possible to study the expression of genes relating to morph differences and craniofacial development in the differentiation of the Arctic charr morphs from Þingvallavatn. For instance, (Ahi et al., 2014) found co-expression relationships between genes involved in craniofacial development in vertebrates, and revealed that different transcriptional dynamics strongly correlated with benthic and limnetic craniofacial morphologies across development (*Timp2* and transcription factors *Ap1* and *Ets2*). The development of craniofacial variation before the onset of feeding across morphs was also investigated by (Kapralova et al., 2015), finding different ontogenetic trajectories among the two benthic charr and the planktivorous charr, reared in the lab from wild caught parents. These patterns were confirmed by a later study on the developmental transcriptome of Arctic charr, which revealed differential expression of at least 7 genes in the developing head that associated with benthic and limnetic morphologies across the four morphs of Arctic charr from Þingvallavatn (Gudbrandsson et al., 2016). The integration of transcriptome data and gene expression patterns in early development later validated these results by observing 2000 genes with differences across developmental stages and across morphs. Although no clear functional enrichment of transcripts between groups was reported from Gene Ontology (GO) analyses (Guðbrandsson et al., 2018), these genes could relate to morph differentiation. Another aspect involved in developmental morphogenesis are developmental regulators, such as micro-RNAs (miRNAs). Despite the fact that only the SB morph from Þingvallavatn and an aquaculture strain were used in (Kapralova et al., 2014), more than 70 miRNAs showed significantly different levels of expression in the two contrasting morphs, indicating that developmental regulators have an important impact on

craniofacial development in benthic and limnetic Arctic charr. Exploring the expression variability of mRNA and miRNA can also provide deep insights into this and other adaptive radiations. A recent publication (Horta-Lacueva et al., 2023) examined the evolution of canalization in terms of expression variability across key developmental points of the in PL and SB charr from Þingvallavatn, using both mRNA and miRNA data. The study found high, but distinct, levels of variability in mRNAs and miRNAs for both intra- and inter-cross embryos, indicating that the two morphs have genetically based differences in gene expression variability across the genome. Interestingly, the hybrids' gene expression variability often followed their maternal variability profiles, which may have an influence in the evolutionary trajectories during morph diverging, likely biasing the effects of hybridisation. Taken together, these results suggest that divergence in gene expression variability evolves rapidly and can occur multiple times across the whole transcriptome in the two small charr morphs from Þingvallavatn. However expression differences do not directly identify the underlying genetic variants that relate to morph differentiation in specific traits.

Besides the advances in transcriptomics, the evolution of immunological genes was additionally studied in the Arctic charr morphs from Þingvallavatn, as it is key to understand adaptation as organisms face novel environmental conditions. One of the most variable genes in vertebrates, crucial for their great capacity of adaptive immune response, is the major histocompatibility complex (MHC). In the Arcrtic charr from Þingvallavatn, (Kapralova et al., 2013) conducted an immunological screen of the SB, PL and LB charr morphs, reporting significant differences in allele frequencies between morphs at the Cath2 and MHCIIα loci.

In the last few years, with NGS (Next Generation Sequencing) and computational resources becoming more accessible, a few studies explored the genetic differentiation among the Þingvallavatn morphs using mostly population genomic approaches (Guðbrandsson et al., 2019). Extracted SNPs from mRNA profiles confirmed genetic differentiation among the LB, SB and PL charr across the whole genome rather than in few islands of differentiation. GO analyses of the genes with strongly differentiated SNPs pointed towards differences in collagen metabolism, odontogenesis, and sensory systems between PL-charr and the benthic morph, indicating that morphological differences attributed between benthic and limnetic morphs have a genetic basis and that specific pathways may have been subject to selection. Another study used a large SNP array with more than 14K SNPs (Brachmann et al., 2022) to test for allelic and genetic at parallelism in the divergence of benthic and limnetic specialisations across multiple Icelandic lakes, including Þingvallavatn. They analysed genetic variations, diet and morphological traits, finding little evidence for parallelism. This indicates that a variable genetic basis of the morphology and dietary preferences that differentiate the benthic and limnetic morphs in this and other lakes in Iceland.

Despite the fact that the studies described above present evidence for a genetic basis behind phenotypic morph differentiation, non-genetic mechanisms also seem to contribute to ecological diversification in the Arctic charr system from Þingvallavatn. Phenotypic plasticity effects seems to contribute to morph differentiation to an extent, when compared with the degree of specialisation seen in other Icelandic lakes (Parsons et al., 2011). Parsons and colleagues exposed juveniles of charr morphs from two lakes to different diets and revealed morphological differences in genetically different benthic and limnetic fish from Þingvallvatn. However, the levels of plasticity were low when compared to other less

specialised populations. These results were further confirmed by a similar plasticity study, admittedly on the same material (Küttner et al., 2013). Besides the more general definition of plasticity, some studies, using different approaches, indicated that maternal effects play an important role in growth and shape across development (Beck et al., 2020, 2022; Eiríksson et al., 1999; Horta-Lacueva et al., 2023; Ponsioen 2020). More recently, a study looking at epigenetic effects across development found differences in DNA methylation profiles between benthic-limnetic ecomorphs, although the cause for these differences is still unknown (Matlosz et al., 2022).

The Arctic charr population in Þingvallavatn has a long and prolific research history, with numerous approaches and technologies employed to unravel the genetic basis of its remarkable ecological differentiation. However, these research outputs represent pieces of a puzzle that is yet to be completed. Furthermore, the incessant development of sequencing methods, the increase of computational power and the release of specialised software and new theoretical models highlight the need to discover the missing pieces. This scenario offers an excellent opportunity to investigate the ecological and evolutionary processes influencing this system.

Focussing on the genetic differentiation among morphs, there are two missing pieces that stand out. First there is a need for a deep study of the genetic architecture underlying the morphological differences related to benthic and limnetic specialisations in the Arctic charr from Þingvallavatn, while including the effects of sexual dimorphism on shape in the equation. Secondly, body size needs genetic dissection. As mentioned before, systems that diverge along an ecological axis divergence also tend to have prominent size differences (e.g. in salmonids reviewed in (Salisbury & Ruzzante, 2022) that have ecological significance and hence deserve attention such is the case of the Arctic charr in Þingvallavatn. In this thesis I aim to tackle the genetic basis of (1) morphological differentiation associated with benthic and limnetic ecologies (Paper I and II) (2) discrete body size differentiation (Paper III)

2 Aims of the thesis

This thesis aims to disentangle the genomic basis of ecological specialisation between the Arctic charr morphs in lake Þingvallavatn. The focus here is on phenotypic traits involved in ecological differentiation, specifically (1) morphology associated with benthic and limnetic ecologies and (2) body size differentiation. To address this, we used sampling of wild populations and rearing of fish in captivity, together with a variety of methodological approaches to assess the nature and complexity of the genetic basis behind ecological specialisation and its patterns (i.e., signals are detected on a genome-wide fashion or in particular genomic locations). The central questions are set forth in ***Table 2*** along with descriptions of relevant sampling (biological model) and respective manuscripts.

Table 2. *Main questions addressed in this thesis, to study the genetic basis of either morphological traits associated with benthic and limnetic ecologies or body size differentiation. The column "biological model" indicates the nature of the fish samples used in the thesis. The "manuscript" column indicates which paper addresses such questions.*

The genetic basis of…	**Question**	**Biological model**	**Manuscript**
Morphological traits related to benthic-limnetic ecologies	Do benthic and limnetic traits have a genetic basis? What are the effects of the onset of sexual maturation on shape?	Laboratory reared F1 offspring from wild PL- and SB-from Þingvallavatn	Paper I
	To what extent do loci with moderate to large effects on phenotype contribute to benthic-limnetic specialisation? Are the loci fixed, or do they cause variation both between and within morphs?	Laboratory reared F2 and backcrosses from the F1 offspring of PL- and SB-from Þingvallavatn	Paper II
Discrete body size differentiation	What are the genetic processes involved in the body size differentiation seen among the four morphs of Arctic charr from Þingvallavatn? What is the role of geneflow in body size differentiation within the lake?	Wild adult specimens collected in multiple locations in Þingvallvatn	Paper III

3 Methods

This project combines a wide variety of techniques in terms of sampling in the wild, experimental design of fish in captivity, phenotypic and genotypic platforms and bioinformatic pipelines.

3.1 In the wild: sampling

For papers I, II and III, fish were sampled in different locations in Þingvallavatn (**Fig. 3**) using two different strategies. For the crosses used in papers I and II the sampling was conducted at the known PL- and SB- charr spawning grounds in the beginning of October of 2015. The collected fish were further used for generating inter- and intra-cross families (**Fig. 4).** The majority of the sampling for paper III, was done during a survey conducted on the known Arctic charr feeding grounds in August and September of 2019. Genetic and phenotypic data were collected at the Institute of Life and Environmental Sciences facilities. For both samplings gillnets were laid overnight (from 10 to 60mm knot to knot).

Figure 3. *Sampling morning in Þingvallavatn. From left to right, Kalina Kapralova, Quentin Horta-Lacueva and Sigurður Snorrasson departing from Mjoanes.*

3.2 In the lab: experimental design

Papers I and II are based in data collected from fish in captivity. Briefly, wild SB and PL morphs were collected in Þingvallavatn in 2015 (F0) to generate crosses of the same morph and reciprocal hybrids. More than 600 fish (F1) were reared in common garden conditions and phenotyped at four time points after fertilisation to explore the genetic component of shape across ontogeny (Paper I). When these fish reached sexual maturation, they were crossed to either each other or to their parental morphotypes to generate two intercrosses (F2) and four backcrosses (BC1), respectively. The progeny (around 450 individuals) was reared in common garden conditions and later used for constructing a high-resolution linkage map and for QTL mapping of benthic-limnetic traits (Paper II). This experimental design conforms one of the major strengths of these thesis given (1) the long generation times of Arctic charr and the challenging, (2) the resource-intensive maintenance procedures, (3) the large number of individuals to be phenotyped and genotyped and (4) the diversity of cross types to enrich the recombination patterns in the linkage map.

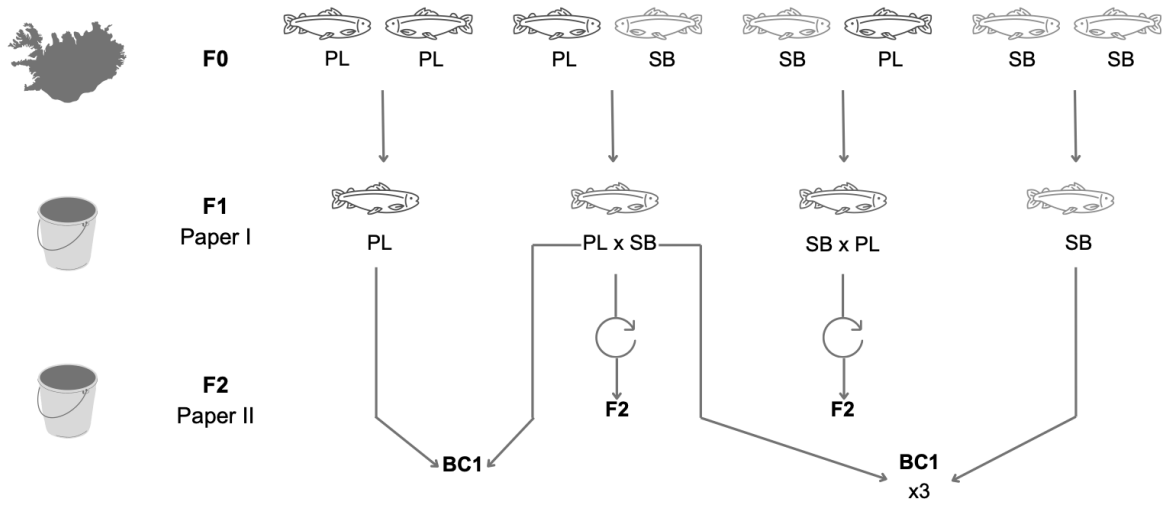

Figure 4. *Crossing design used for Papers I and II. The Iceland outline represents that samples were taken from the wild. The buckets represent that fish were reared in captivity in common-garden conditions.*

3.3 Phenotyping

The phenotyping platforms were common for all the charr specimens used in this thesis, regardless on the charr being directly caught in the wild or sampled from the families in captivity. If the charr were sampled directly from Þingvallavatn, sexually mature fish were classified to morph following (Snorrason et al., 1989). For all specimens, the fork length and weight of the fresh fish was measured, and pictures were taken on their lateral view for further phenotyping. Fin and/or muscle tissue samples were taken for DNA extraction. The pictures were later used for landmarking and geometric morphometrics analyses, although simple linear measurements were also extracted from interlandmark distances for paper II. tps.dig (F. Rohlf, 2015) and r/Stereomorph (Olsen & Westneat, 2015) were used for landmarking, while r/geomorph and r/RRPP (Adams et al., 2024; Baken et al., 2021; M. L. Collyer & Adams, 2018) were used for analysis of shape variation.

3.4 Genotyping

Analogously to phenotyping, the genotyping platforms were consistent for all charr specimens included in papers II and III. DNA extraction was conducted on muscle or fin tissue using a standard phenol-chlorophorm protocol (Taggart et al., 1992), followed by a custom ddRAD sequencing library preparation protocol based on (Lagunas et al., 2023). All samples were sequenced with the same technology (i.e., one library per lane in HiSeq X-ten at BGI Hong Kong).

3.5 Bioinformatic pipelines

Data pre-processing, demultiplexing, alignment, SNP calling and filtering tools were common to all sequenced individuals from papers II and III. For paper II, the resulting reads and pedigree information from the experimental design were used for constructing the linkage maps, and QTL mapping was conducted one trait at a time and one family at a time. For paper III, the resulting reads and morph information were used for traditional population genomics analyses and backwards simulations.

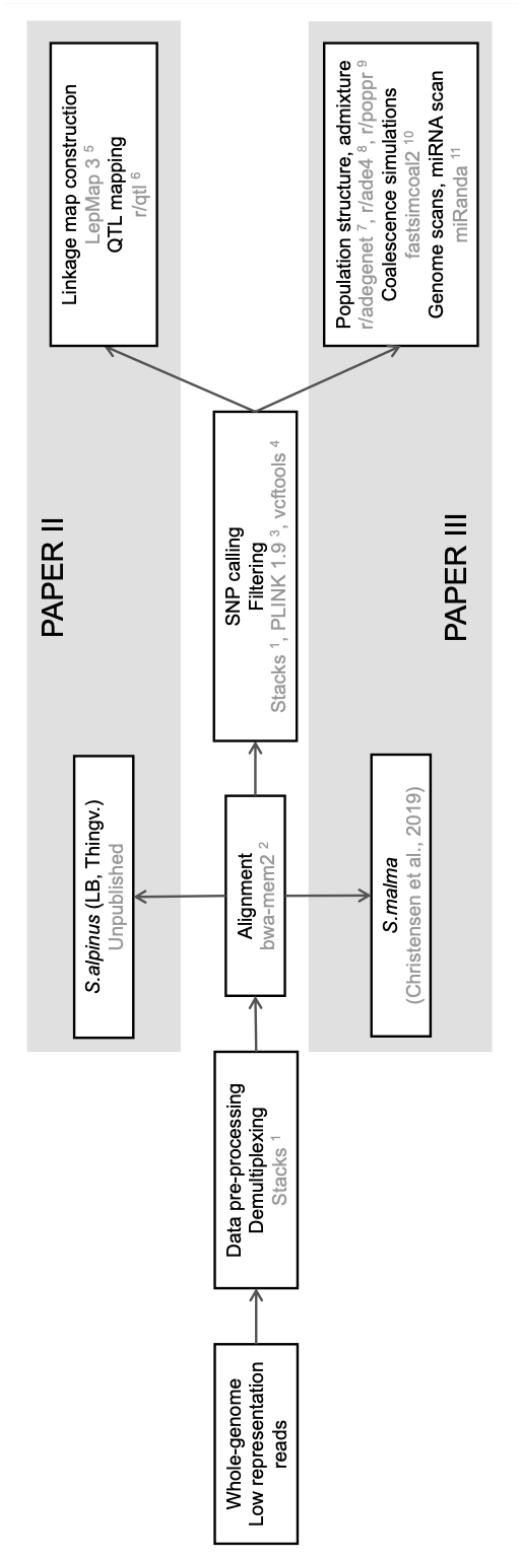

Figure 5. *Summary of the bioinformatic pipelines used for the genetic data in papers II and III.* [1]*Catchen et al., 2011, 2013,* [2]*Md et al., 2019,* [3]*Purcell & Chang 2015,* [4]*Danecek et al., 2011,* [5]*Rastas et al., 2017,* [6]*Broman et al., 2003,* [7]*Jombart 2008,* [8]*Thioulouse et al., 2018,* [9]*Kamvar et al., 2014,* [10]*Excoffier et al., 2013, 2021,* [11]*Enright et al., 2003.*

4 Results

In **paper I,** the benthic and limnetic morphs of Arctic charr from Þingvallavatn were used to explore interactions between ecological diversification and sexual dimorphism. A long-term common-garden experiment was conducted, where I analysed shape variation throughout the development of intra- and inter-morph crosses of benthic (SB) and limnetic (PL) charr from the lake. My findings revealed that shape differences between ecomorphs and sexes have a genetic component. Before sexual maturation, shape differences were influenced by cross type and were associated with adaptations to benthic and limnetic habitats, such as shorter lower jaws and rounder snouts in benthic charr, and evenly protruding and pointier snouts in limnetic charr. Reciprocal hybrids exhibited intermediate, transgressive, and/or maternal morphologies. However, after sexual maturation, the morphological differences between sexes became more pronounced than those among cross types. Overall, these results highlight the complex and dynamic nature of the relationship between ecological diversification and sexual dimorphism throughout ontogeny, emphasizing the value of long-term common-garden experiments in studying morphological dynamics across different evolutionary contexts.

After confirming the genetic basis of shape variation in the first generation, and targeting a time point during ontogeny where differences between benthic and limnetic morphs were the greatest (i.e., 18 months after fertilisation for F1), the progeny of F2 and BC1 crosses was used to map those traits onto the genome. In **paper II** I constructed a linkage map and conducted QTL mapping. The phenotypic matrix included both targeted (i.e., selected linear measurements classically associated with adaptations to benthic and limnetic habitats) and not targeted traits (i.e., PC scores of body shape variation). Given the important effects of sex on shape explored in paper I, sex was added as a covariate in the QTL scans and QTL models. The QTL mapping analyses confirmed that a substantial number of morphological traits related to benthic or limnetic feeding have a genetic component. I found that those morphological traits were mostly explained by a single QTL with modest to high percentages of explained phenotypic variance, likely in combination with other QTL of small effects. The most consistent QTL detected across families and/or locations explained shape differences related to the relative size of the head, maxilla shape, and peduncle depth.

In papers I and II I studied the genetic basis of shape variation focusing on the benthic-limnetic ecological axis of divergence. For that I looked at recombination within known pedigrees in controlled conditions. In **paper III**, the survey samples of the four morphs from Þingvallavatn were used to explore the historical and genetic basis of body size, another important axis of divergence that defines the ecomoprh diversity within the system. For this, population genomics methods, genome scans and miRNA scans were employed. Genetic mechanisms, likely influenced by introgression and standing genetic variation, were found to contribute to body size differentiation. Notably, highly differentiated genomic regions were shared between the small and large morph pairs, including a region containing the glypican-6 (*gpc6*) gene, which is highly conserved in vertebrates and plays a role in cell proliferation and growth. Furthermore, miRNA target sites located in the 3'-UTR of *gpc6* differed across morphs. These findings suggest that the genetic mechanisms underlying body size differentiation are complex and interwoven with phenotypic plasticity and maternal effects.

5 General discussion and research outlook

The main aim of this thesis was to disentangle the genomic basis behind traits involved in ecological specialisation and its patterns (i.e., signals are detected on a genome-wide fashion or in particular genomic locations). In all three papers, I provide evidence for a genetic basis behind the phenotypic traits under study (i.e., body size and shape), using an indirect approach (rearing individuals in common garden conditions and studying shape variation, in paper I), conducting QTL mapping (using families in captivity in paper II) and2 performing genomic scans within a population genomics framework (collecting specimens from the wild, in paper III).

5.1 Transversal threads across the three papers

There are a few conceptual threads that traverse the three papers in this thesis, and whose relevance has not been discussed in each specific paper. These threads comprise (1) the complexity of the genomic architecture of traits involved in ecological specialisation, (2) the often-overlooked effects of sexual maturation on shape, (3) the importance of studying trait covariance and (4) the ontogenetic and evolutionary allometry component of shape. These threads and its relationship with each paper are represented in **Fig. 6**.

Papers II and III touch upon the complexity of the genomic architecture behind traits that may be involved in ecological specialisation. Briefly, there are two non-mutually exclusive hypotheses: that the genetic underpinnings of a phenotypic trait are driven by (1) a single or a few genomic regions of large effects or (2) multiple genomic regions of smaller effects. Although these hypotheses are only formally tested in paper II, suggesting a mixed, hierarchical genetic architecture of traits related to benthic and limnetic specialisations, paper III seems to point towards a similar direction, where only one highly differentiated region of the genome was shared between large and small pairs, and seemed to be at least partially responsible for body size differentiation within the lake. However, these results should only provide a general indication of the nature of the genomic architecture behind a variety of phenotypic traits that are connected in different ways, and the two studies should not immediately be compared due to the low-representation sequencing method used, the different methodological approaches applied, and the variable genetic and molecular mechanisms involved.

The effect of sexual dimorphism on shape, discussed in papers I and II, had been slightly overlooked in studies about charr in Þingvallavatn (but see (Jónsdóttir et al., 2024) where no significant differences between sexes were found in head bone shape). In paper I the onset of sexual maturation was found to have a profound impact in body shape variation, heavily overriding those morphological aspects attributed to benthic or limnetic specialisations. The caveat is that these fish were grown in the laboratory, and body shape of these fish at the same age in the wild remains to be investigated. This urged us to sample

the second generation (F2 and backcrosses) at a time point where the effects of sexual dimorphism on shape variation were not that substantial. However, as expected, significant effects of sex on different phenotypic traits were found in the different families, and sex was always included as a covariate in all QTL scans and models, even if that required decreasing sample size per family and thus statistical power. Even if we do not observe evident sexual dimorphism in the wild small morphs from Þingvallavatn (i.e., SB and PL) here I highlight the importance of taking the effects on sexual dimorphism into consideration for further studies. A third invisible thread in the study of shape variation concerns trait correlation. While no direct linear measurements were taken for paper I, analyses of trait correlations across time points (paper I) and generations (paper II) would provide valuable information about trait covariance, which would expand on previous studies on hybrid incompatibilities and ultimately, barriers to reproductive isolation (Horta-Lacueva et al., 2021).

The last thread emphasises the importance of growth as a key factor for understanding evolutionary change in fish evolution. Specifically, in paper I different phenotypic patterns among the intra- and inter-morph crosses were found at the different points during development, highlighting the importance of ontogenetic allometry (i.e., how shape varies across development (Klingenberg & Zimmermann, 1992)). A similar trend was observed in paper II, where shape covaried with size in different ways in the different families, emphasizing that, not only ontogenetic growth but also the genomic composition of the family, and likely fish density throughout the rearing, also influence shape via ontogenetic growth (i.e., ontogenetic allometry). Furthermore the last paper touches upon the concept of how ontogenetic allometry and evolutionary allometry are interconnected. Briefly, while the PL and the PI charr from Þingvallavatn belong to the same genetic population, the PI population has both the opportunity and the capacity to grow larger and undergo an ontogenetic shift towards a piscivorous diet, as long as there is some introgression from the LB charr genome. The PI charr is a unique ecomorph that obtains its characteristics likely from the combination of the ontogenetic shift and to an extent, some sort of genetic predisposition. In sum, the allometric component of shape across either ontogeny or evolutionary history can function as a source of phenotypic diversity, facilitating the process ecological diversification.

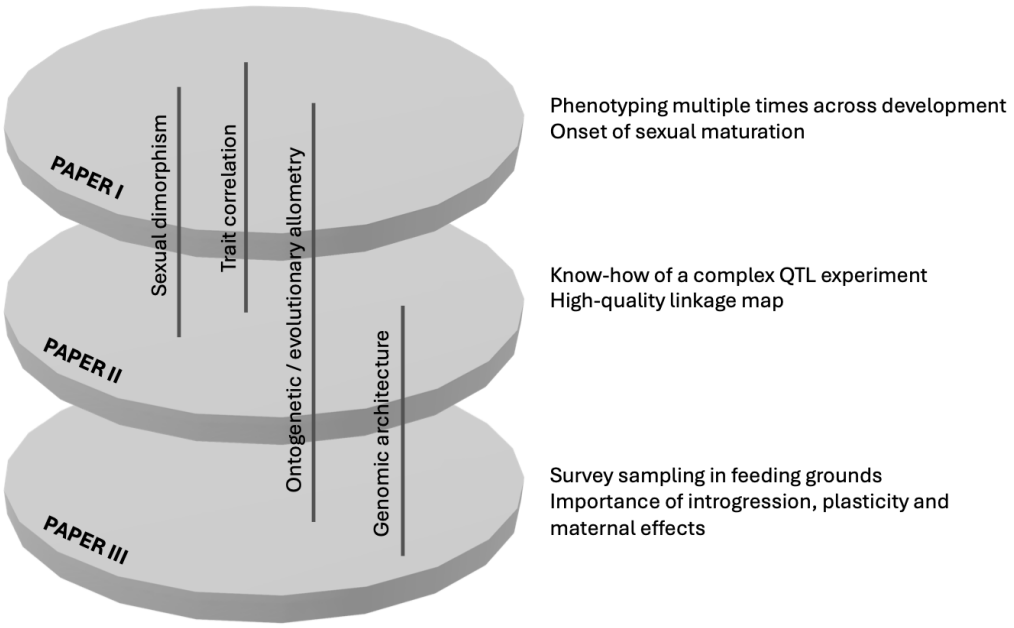

Figure 6. *The grey disks represent the three papers included in this thesis. The vertical dark blue lines depict the common transversal threads discussed. The text on the right highlights the aspects from each paper that should be taken into consideration for future search on this and other salmonid systems.*

5.2 From the lab to the wild

The strengths of this thesis are multiple. First, the Arctic charr from Þingvallavatn is an excellent system to study evolutionary processes, as it was discussed both in the Introduction chapter and along the three papers (and references therein). Second, sampling both from the wild and from families reared in captivity allowed for an integrated overview of the evolutionary processes occurring in Þingvallavatn (i.e., morphological, developmental and genetic differentiation between morphs). A good representation of the four charr morphs in the lake was possible thanks to the survey sampling approach, rather than limiting the sampling efforts to the known spawning locations. On the other hand, the generation of the QTL families itself, and the information extracted from them is likely the most valuable aspect of this project. Experimental evolutionary research tends to use small bodied model organisms with short generation times such as microorganisms, plants, insects or even small vertebrates such as zebrafish or sticklebacks (e.g., (Burke et al., 2010; Exposito-Alonso et al., 2018; Lenski, 2017). This allows for a stricter control of the experimental conditions and for an increase in the number of generations and the diversity of cross types. Paper II is, to my knowledge, the first study that conducted a QTL experiment in Arctic charr to the second generation, in common garden conditions and including a variety of cross types, moreover, using a relatively large organism with long generation times and extremely delicate breeding and rearing requirements. Thanks to this massive effort, a high-quality linkage map is now available for Icelandic Arctic charr, whose uses can significantly advance our knowledge on the genetic basis of ecological specialisation in salmonid systems.

While the QTL experiment, map construction and QTL mapping are an important asset to evolutionary research, one main limitation stands out from this thesis, and is the lack of formal integration between the wild data from paper III and the captivity data from papers I and II, or "how to go from the wild to the lab, and vice-versa". One way to address this would, for instance, involve conducting GWAS on the wild Þingvallavatn population to first investigate how often genomic regions with signatures of selection also associate with phenotype. We would later proceed with detecting overlapping genomic regions among the genomic screen, the GWAS signals and the QTL mapping signals. This approach would yield to valuable results as we are linking the genomic architecture of traits traditionally associated with benthic and limnetic adaptations to potential adaptive peaks that may have occurred in the wild (e.g. (Albertson et al., 2014; Laporte et al., 2015; Rogers & Bernatchez, 2005). Despite the fact that ddRAD-seq is a cost-efficient approach to genomic data analysis, this sequencing method has the obvious limitation of low genomic coverage. This approach is not optimal for detecting signal overlap from different types of analyses, hence a sequencing method that allows for a higher representation of the genome would be preferred in future studies. Moreover, if the effect sizes of a genotype on a phenotype are small and selected upon, we will not be able to detect signatures of adaptation when assuming the classical "strong selection (i.e., hitchhiking)" model. To overcome this challenge, one could apply a "polygenic approach", which involves looking at QTL covariance with random forest modelling (Laporte et al., 2015).

5.3 Final thoughts

There is a growing body of research exploring the genetic and molecular foundations of ecological specialization. Some systems have become textbook examples, not only due to their suitability for studying evolution but also because of the novel, comprehensive methodologies developed to unravel evolutionary processes at various scales, from evolutionary histories to the identification of genes driving adaptation and their regulatory mechanisms (Abzhanov et al., 2004; Arnegard et al., 2014; Kocher, 2004; Reid et al., 2021). While Arctic charr systems are often cited as iconic examples of resource polymorphism, and their ecology is extensively documented, genetic research has progressed more slowly compared to other adaptive radiations for several reasons: the large genome size makes whole genome sequencing (WGS) costly, complex chromosomal rearrangements pose difficulties in alignment and dealing with paralogous sequence variants, the species' delicate nature makes them challenging to rear in captivity due to long generation times, large sizes, and extreme susceptibility to temperature fluctuations, and the lack of parallelism across systems, which indicates a variable genetic basis of traits and hence complicates streamlined research objectives.

Despite areas that could be refined in this thesis, its scientific value is evident through advances in morphometrics, population genetics, and quantitative genetics. Notably, the linkage map presented in paper II stands as a valuable resource for future quantitative studies. The boundary between model and non-model organisms is often indistinct, and I believe that Northern freshwater systems, such as the Arctic charr from Þingvallavatn, hold the potential to provide novel insights into evolutionary processes at a microevolutionary scale. This work significantly contributes to our understanding of ecological diversification and opens avenues for further research in this field.

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PAPER I

Evolutionary Biology
https://doi.org/10.1007/s11692-022-09592-y

RESEARCH ARTICLE

The Dynamic Ontogenetic Shape Patterns of Adaptive Divergence and Sexual Dimorphism

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Received: 30 March 2022 / Accepted: 25 December 2022

Abstract
The interplay between ecological diversification and sexual dimorphism has been largely overlooked in the literature. Sexually dimorphic species which are also undergoing adaptive radiations are ideal for filling this knowledge gap. The Arctic charr in lake Thingvallavatn is one such system: it is a sexually dimorphic species which has recently diverged along the benthic-limnetic ecological axis. In a long-running common-garden experiment we studied the shape variation throughout ontogeny of intra- and inter- morph crosses of benthic and limnetic charr from the lake. We found that shape differences between ecomorphs and sexes had a genetic component. Prior to the onset of sexual maturation, shape differences were attributable to cross type and were related to adaptations to benthic and limnetic niches, i.e., shorter lower jaws and rounder snouts in the benthic and evenly protruding snouts and pointier snouts in the limnetic. Reciprocal hybrids showed intermediate, transgressive and/or maternal morphologies. However, after the onset of sexual maturation larger morphological differences occurred between sexes than among cross types. Taken together, our results demonstrate that the interplay between ecological diversification and sexual dimorphism is complex and dynamic throughout ontogeny, and that long-term common garden experiments are immensely valuable for studying shape dynamics in different evolutionary scenarios.

Keywords Ontogeny · Adaptive divergence · Sexual dimorphism · Arctic charr · Geometric morphometrics

Introduction

Adaptive radiations provide unique insights into the origins of polymorphic populations (Losos, 2010; Schluter, 2000). Often, species undergoing adaptive divergence also exhibit sexual dimorphism (Gillespie, 2004; Grant & Grant, 2002; Jones et al., 2012; Losos & Schneider, 2009; Meyer, 1993; Snorrason & Skúlason, 2004). However, the interplay between traits related to adaptive divergence and secondary sexual traits has been largely overlooked (but see Bolnick & Doebeli, 2003; Butler et al., 2007; Berra, 2001; Cooper et al., 2011).

Resource polymorphism is a major driver of adaptive divergence where organisms exploit different resources, leading to phenotypic and/or genotypic differentiation (Skúlason et al., 1993; Smith & Skúlason, 1996). In teleosts, common cases of resource polymorphism occur along benthic-limnetic ecological axes (Seehausen & Wagner, 2014). Phenotypes associated with such divergence are often adaptive: fish occupying benthic ecological niches usually have subterminal jaws, blunt snouts, deep bodies, smaller eyes and less gill rackers, whereas fish occupying the limnetic zone have terminal jaws, pointed snouts, slender bodies, bigger eyes and more gill rackers (Berra, 2001; Blake et al., 2005; Hulsey et al., 2013; Sandlund et al., 1992; Snorrason et al., 1994). In recent adaptive radiations, the interplay between traits associated with benthic-limnetic adaptations and sexual dimorphism is best studied in sticklebacks (e.g,:Aguirre et al., 2008; Berner et al., 2010; Cooper et al., 2011; Kitano et al., 2007; McGee & Wainwright, 2013; Reimchen & Nosil, 2004). Although very few examples exist in salmonids (Bjoeru & Sandlund, 1995; Janhunen et al., 2009) these studies already paint a complex and dynamic picture of sexual dimorphism throughout the lifecycle.

Recently diverged populations with relatively simple demographic histories and that are still hybridising facilitate the study of the genetic basis of adaptive traits.

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Published online: 17 January 2023

Hybridisation between or within closely related species will lead to a breakdown of coadapted alleles, heterosis and/or non-additive effects (Dobzhansky, 1936; Ackermann et al., 2006; Arnegard et al., 2014), and see (Bell and Travis, 2005) which will result in intermediate, transgressive and/or parental-like traits (e.g., Seehausen, 2004; Elgvin et al., 2017; Skúlason et al., 1989, respectively). Yet, other phenomena such as genotype x environment interactions and phenotypic plasticity can have strong confounding effects. Despite their logistic inconveniences, common garden experiments are a conceptually straightforward solution to overcome these effects, as they standarise for environmental cues (Villemereuil et al., 2016). Moreover, long term common garden experiments will allow us to gain insight into how the genetic component of morphological traits unfolds throughout ontogeny (e.g., Vučić et al., 2019).

In the present study, we used a long-running common garden setup, encompassing the onset of sexual maturation, to determine the factors responsible for the genetically based shape variation during the post-embryonic ontogeny of a textbook example of a polymorphic species: the Arctic charr in lake Thingvallavatn (Iceland). In Thingvallavatn, Arctic charr has diverged along the ecological benthic-limnetic axis in four ecomorphs (two benthic and two limnetic). For this study, we focused on one benthic (small benthic, SB) and one limnetic (planktivorous, PL) morph, with strong phenotypic differences. Despite recent evolutionary divergence, their overlapping spawning periods (Jonsson et al., 1988) and comparable sexual maturation times (Snorrason et al., 1989), gene flow between SB and PL is rather low (Brachmann et al., 2021; Jonsson et al., 1988; Kapralova et al., 2011; Skúlason et al., 1989), but it is still possible to generate hybrids in captivity. Offspring of wild SB and PL morphs from the lake and their reciprocal hybrids were reared in common garden up to 36 months and phenotyped at four time points during ontogeny.

The aim of this study was to investigate the factors driving genetically based shape variation throughout the ontogeny of two Arctic charr ecomorphs and their hybrids. We hypothesized that (1) traits associated with benthic-limnetic adaptations will have a non-additive genetic effect, (2) the effective load of morphs will increase during ontogeny, and (3) sexually dimorphic traits will only be detectable after the onset of sexual maturation.

Material and Methods

Study System

Thingvallavatn is a post-glacial lake situated in south-western Iceland, colonised by Arctic charr (*Salvelinus alpinus*) less than 12 K years ago. After this single colonisation event, Arctic charr diverged into four ecomorphs along the benthic-limnetic axis, representing a text-book case of resource polymorphism. The diverged morphs resulted in two bottom-feeders (i.e., a small and a large benthic, SB and LB) and two limnetic-feeders (i.e., a planktivorous and a piscivorous morph, PL and PI) (Guðbrandsson et al., 2018; Jonsson et al., 1988; Kapralova et al., 2011, 2013; Malmquist et al., 1992; Sandlund et al., 1992; Snorrason et al., 1989).

Traits associated with benthic-limnetic adaptations also occur throughout ontogeny in the form of an ontogenetic shift. All Arctic charr ecomorphs start feeding on the benthos at juvenile stages (Parsons et al., 2011; Skúlason et al., 1989), and limnetic morphs migrate towards pelagic areas in order to feed on plankton (planktivorous morph, PL) or other fish (piscivorous, PI), whilst benthic morphs retain these juvenile-like traits to continue feeding on the benthos (Skúlason et al., 1989).

Data Collection

Generation of Crosses and Rearing

Wild adult specimens of the planktivorous (PL) and small benthic (SB) morphs were collected by laying gillnets overnight at a spawning site shared by both morphs (Svínanesvík, 64°11′24.6"N; 21°05′40.5"W) in the beginning of October 2015. A total of 31 mature specimens were crossed either within the same morph (i.e., intra-morph crosses, PLxPL and SBxSB) or reciprocally with the alternative morph (i.e., reciprocal hybrid crosses, PLxSB and SBxPL, always ♀ x ♂), generating 19 full-sibling families (see crossing design in Supplementary material (SM), table S1). Fertilised eggs from each family were placed in separate mesh cages in an EWOS hatching tray (EWOS, Norway) at 4.1 ± 0.2 °C in the aquaculture facilities of Hólar University College, Sauðárkrókur, Iceland. After hatching and first feeding, families were transferred into separate buckets (35 cm deep, ∅ 29 cm), connected to the same running water flow and fed manually with commercial dry pellets in the same way to ensure common feeding conditions. Fish were phenotyped throughout ontogeny at 12, 18, 24 and 36 months after fertilisation. At each time point, specimens were anaesthetized with appropriate dose of 2-phenoxyethanol (Pounder et al., 2018). At 12 months specimens were only photographed and no individual information was collected. Those families with a larger number of individuals were separated into two buckets to control for density. At 18 months all fish were PIT- tagged, weighted, photographed and placed together in two aquaculture tanks with similar densities. At month 24, fish were again weighted and photographed, and at month 36 they were photographed, and their sex determined if they had reached sexual maturation. Since fish were PIT- tagged at month 18, individual information collected at later time

points could be traced back. However, no sex data were available for month 12. Mortality throughout the experimental setup was low (less than 10%), and higher levels of mortality were not attributed to any specific cross type or family.

Photographing

Each individual was photographed at 12, 18, 24 and 36 months. Photos were taken on their left lateral side with a fixed digital camera (Canon EOS 650D and 100 mm macro lens) along with a ruler for scaling. Fish tend to naturally bend as they are positioned on a rigid flat surface while the photos are being taken, which may confound biologically meaningful shape variation (Valentin et al., 2008). We corrected for potential bending effects by placing 5 equidistant landmarks along the lateral line of each fish and implementing the unbending tool in tpsUtil (Rohlf & tpsDig, 2016).

Landmarking

We placed 16 landmarks and 4 semilandmarks on each photo using tpsDig (version 2.26, (Rohlf & tpsDig, 2016)) (Fig. 1), following (Adams & Huntingford, 2004; Parsons et al., 2010) and see landmark description in SM2. For scaling, two additional landmarks were placed on a ruler and were removed before performing Procrustes superimposition. Landmarking of the data was conducted by the same person. Thirty random individuals from different cross types and families were landmarked three times, obtaining a high repeatability ($p < 0.05$) which ensured the robustness of the data.

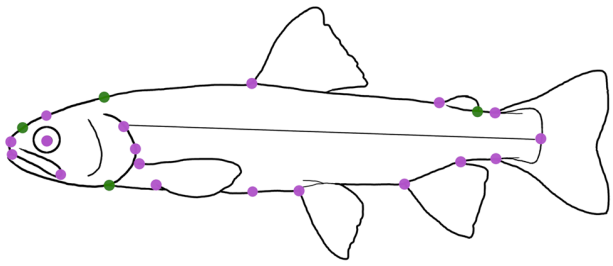

Fig. 1 Landmark (violet) and semilandmark (green) configuration used for geometric morphometrics analyses. Landmarks taken for scaling and unbending are not shown (Color figure online)

Analysis of Shape

Raw coordinates in.tps format were imported in R and subsequent analyses were conducted with the geometric morphometrics package *geomorph v4.0.0* and *RRPP v1.0.0* (Baken et al., 2021; Collyer & Adams, 2018, 2021). Outlier examination was conducted by looking at Procrustes distance of each landmark configuration to their mean shape, grouped by month and cross type. Configurations falling on the upper quantile of the distribution represented 2.18% of the full data set and were inspected individually. These few specimens showed either landmark displacements or slightly open jaws, were considered outliers and were removed from the final dataset. After outlier removal, Partial Generalized Procrustes Superimposition was performed on raw data and the resulting Procrustes coordinates were used in downstream analyses (see Table 1 for number of individuals used at each time point). During Procrustes superimposition, each landmark configuration is translated, scaled and rotated to minimise shape differences among them.

Centroid size (the square root of the sum of squared distances of the landmarks to the centroid) is extracted during scaling and it is often used as a proxy for body size (e.g., Parsons et al., 2010; Kapralova et al., 2015). This may not be the case for our dataset as Arctic charr have fusiform bodies and we unevenly positioned the landmarks along the shape outline (a considerable proportion of the landmarks were on their heads) (Collyer et al., 2020). As body weight information was missing for months 12 and 36, we tested whether centroid size could be used as a reliable indicator for body weight for our dataset. A high correlation between centroid size and body weight (98.6% (Pearson's correlation coefficient, $p < 0.05$) on the log–log regression of both variables) for a subset of individuals ($N_{18\ months} = 653$, $N_{24\ months} = 595$) confirmed that centroid size can be used as a proxy for total body size in our study.

Statistical analyses were performed at two different levels: (1) on a full data set of specimens to study the dynamic patterns of shape variation across four time points (i.e., month 12, 18, 24 and 36) with no sex information and hence

Table 1 Number of individuals used for geometric morphometrics analyses (outliers removed) per cross type and sex at each time point

	Month 12				Month 18				Month 24				Month 36			
	F	M	NA	Total	F	M	NA	Total	F	M	NA	Total	F	M	NA	Total
PLxPL	–	–	–	118	44	60	47	151	43	57	28	128	42	57	20	119
PLxSB	–	–	–	267	65	119	51	235	64	112	42	218	64	118	40	222
SBxPL	–	–	–	244	73	89	72	234	68	89	60	217	70	84	59	213
SBxSB	–	–	–	26	4	10	19	33	4	10	18	32	3	9	16	28
Total	–	–	–	655	186	278	189	653	179	268	148	595	179	268	135	582

heterogeneity in the sexual maturation state of the specimens and (2) on specimens which reached sexual maturation during the experimental setup and thus individual sex information could be traced back to month 18 and studied across time. For both datasets, mixed-model MANOVAs were performed with *geomorph::procD.lm* to examine the effects of *log(Csize), month, sex* (if applicable) and *cross type* with nested families (*cross/family*) (including all possible interactions) on *shape* (i.e., and here after, Procrustes coordinates). The *RRPP::pairwise* function was used to assess differences in shape between group means and variances (the latter as a proxy for morphological disparity). These analyses were performed at each separate time point and also pooling together individuals phenotyped at different time points. To study ontogenetic trajectories of sexual dimorphism (months 18, 24 and 36) we performed Phenotypic Trajectory Analysis (PTA) in *geomorph*. Due to unbalanced number of sexed individuals among cross types and families (see Table 1, and SM1 Table 1), we decided to pool the four cross types together and study intraspecific sex trajectories as a whole. We explored pairwise differences in length (i.e., amount of shape change), directionality, shape and location in the morphospace between both trajectories. For the same data set, MANOVAs and pairwise tests were performed separately on the different time points. We used *geomorph::plotAllometry* for visual interpretation of static allometry patterns and Homogeneity of Slopes (HOS) tests were conducted by comparing models of common (*shape ~ log(Csize) + sex*) versus unique allometry (*shape ~ log(Csize) * sex*) statistically in order to decide whether to correct for static allometric effects within each time point.

Visualisation

Principal Component Analyses (PCA) on Procrustes coordinates (Adams et al., 2013) were conducted for visualisation of shape variation at each level. Additionally, we used wireframes to explore shape changes in landmark configurations at the extremes of the most relevant eigenvectors (Bookstein, 1989). Ontogenetic trajectories were plotted onto the first two principal components based on the covariance matrix of group means. Trajectories connect mean shape estimates at the different time points for each sex or cross type.

Results

Benthic and Limnetic Traits Have a Genetic Component

We found significant differences in mean shape between PLxPL and SBxSB within each time point ($p < 0.05$, see mean pairwise comparisons in SM4) when fitting explicit reduced models (*shape ~ 1 + log(Csize)*). However, we did not see significant differences in shape variance (SM4).

Except for month 36, either the first principal component, the second or both separate the PLxPL and SBxSB crosses (Fig. 2). Wireframes representing the predicted shapes at the extremes of those PCs are consistent among each other: intramorph SB crosses tend to have deeper bodies, deeper heads and rounder snouts compared to intramorph PL crosses, which have more fusiform bodies, narrower heads and pointier snouts. However, the position of the reciprocal hybrids in the morphospace is not as consistent, indicating that their shape variation is highly dynamic throughout ontogeny. Specifically, at month 12 (Fig. 2A) reciprocal hybrids laid in the middle, but they did not completely overlap with each other, being the maternal morph closer to each of them. At month 18 (Fig. 2B), a substantial proportion of hybrids showed transgressive (i.e., outside of the parental range) phenotypes at high values of PC1, the main axis of variance (29.95%). The rest of the hybrid specimens at month 18 laid between their parental phenotypes. At month 24 (Fig. 2C) the pattern again switched, showing that hybrid shape variation was mostly contained within their maternal morphospace. Nevertheless, the positioning of reciprocal hybrids was not symmetrical: SBxPL shape variability is particularly reduced compared to PLxSB, which completely overlapped with PLxPL, and also expanded towards SBxSB and SBxPL's morphospaces.

Although cross type was found to have a significant effect on shape at month 36 (Fig. 3) (and overall shape differences were significant between all pairs (SM4, table S4.10)), no clear cross type clustering was found when examining the first ten principal components (Fig. 2D).

Regarding allometry, only in month 12 the null hypothesis of homogeneity of slopes between cross types was not rejected ($p = 1.000$). However, we decided not to correct for common allometry not only for easier comparison among time points, but also because shape variation due to change plays an important role during ontogeny.

Sexual Dimorphism is Present Before Sexual Maturation Occurs

When looking at the reduced dataset that includes sex, the effect of sex alone on shape was significant both when all the individuals from different time points are pooled together (SM3) and at each separate time point, although the effect was particularly important at months 24 and 36 (Fig. 3, SM3).

We focused on sexually mature individuals at month 36 to study the maximum dissimilarity in shape between males and females. The shape variation along PC1 (Fig. 4) was explained by differences between sexes, with certain point overlap between the two clusters. The wireframes depicted

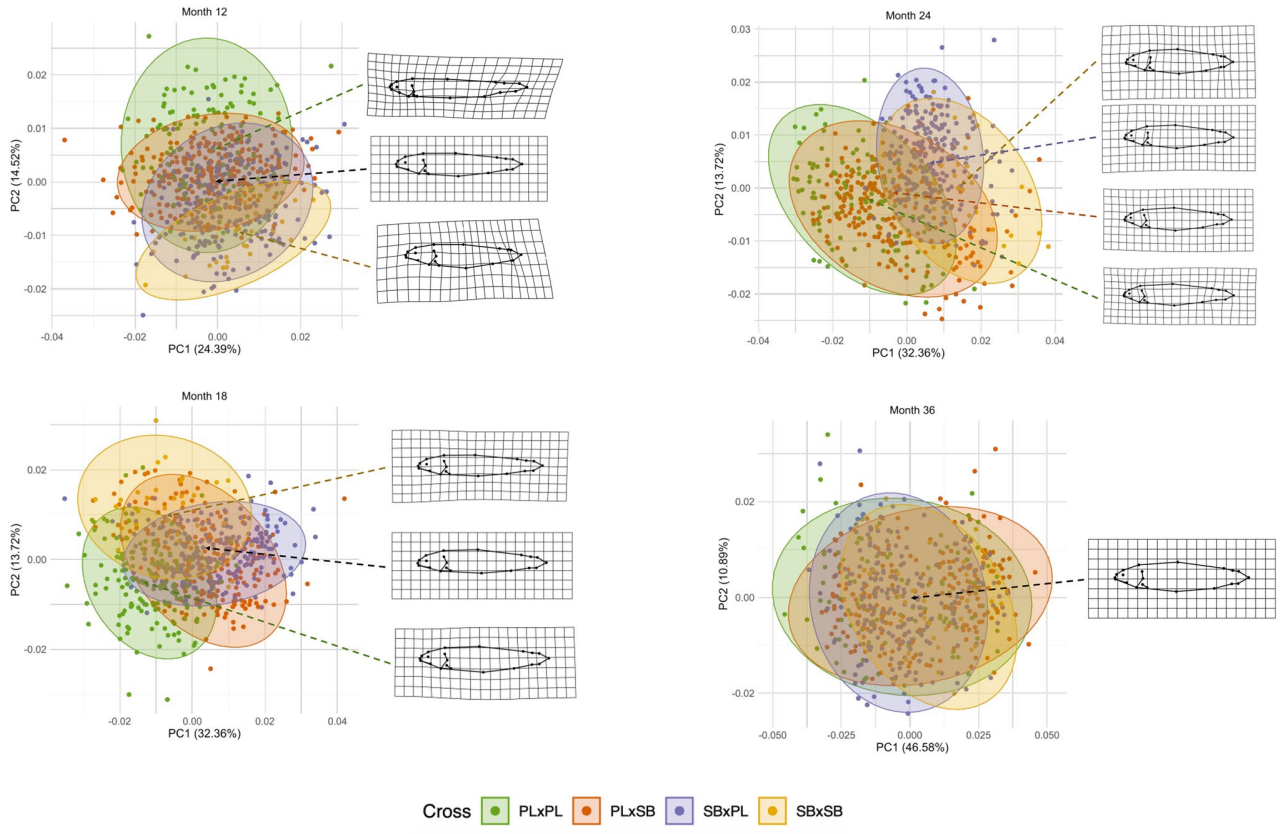

Fig. 2 Two first principal componensts of separate principal component analyses of months 12 **(A)**, 18 **(B)**, 24 **(C)** and 36 **(D)**. Each point represents one individual and shaded areas depict 95% confidence ellipses. Grids represent the predicted mean shape of each cross. The observed shape changes in the grids are in respect to the grand mean of all individuals per time point. Two morphs are represented by one grid when their mean shapes were not significantly different from the grand mean

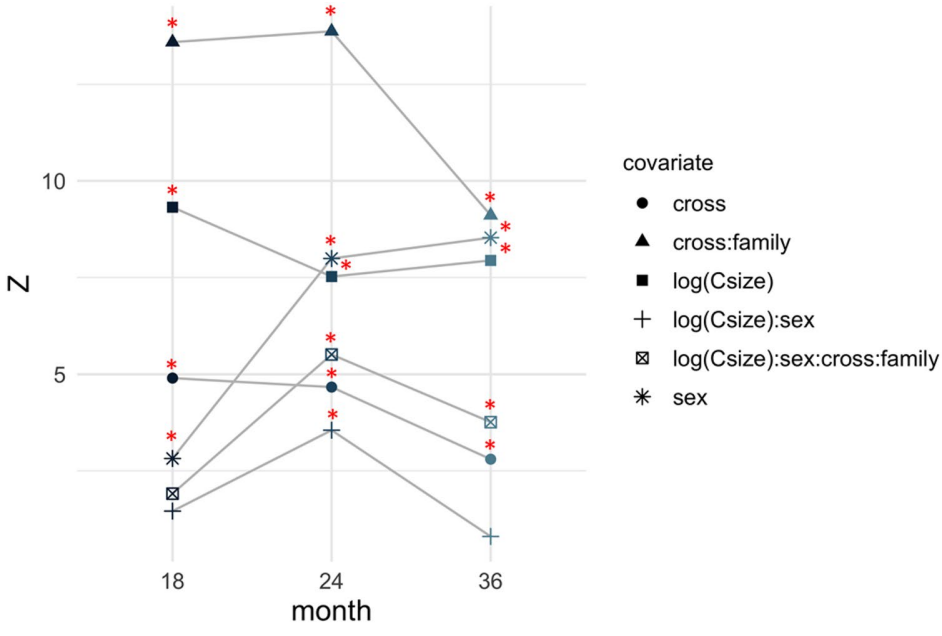

Fig. 3 Relative loads of significant covariates in independent MANOVAs at month 18, 24 and 36, represented by Z scores and p-values < 0.05 (asterisks) (Color figure online)

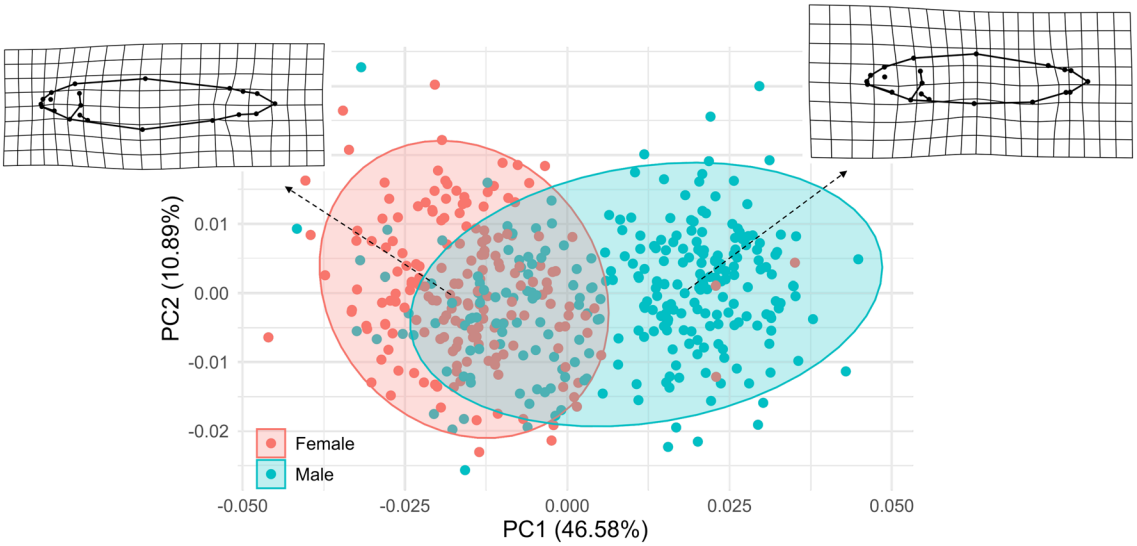

Fig. 4 Principal component analysis of Procrustes coordinates of sexually mature specimens at month 36. Each point represents one individual. Shaded, 95% confidence ellipses for each sex. The grids represent shape changes in mean shape compared to the grand mean in females and males

that females had substantially smaller heads compared to males, more stream-lined bodies and slightly longer caudal peduncles. Compared to females, males seemed to have pointier snouts, but no differences in the position of upper and lower jaw were detected. However, we were not able to determine to what extent traits associated with sexual dimorphism are confounded due to the effect of traits associated to benthic-limnetic adaptations, which do not lay at PC extremes and thus their variation cannot be represented in a PCA. Despite the fact that it was not possible to study sexual dimorphism within cross type due to data imbalance (especially in the case of the SB crosses), we examined whether intra-morph crosses or reciprocal hybrids clustered towards the "most male or female" morphospace, but found no clear structure (SM6 for visualisation). Another confounding factor may be the heterogeneous sexual maturation time (i.e., some individuals were not mature yet, and thus not sexed and not used for sexual dimorphism analysis) and/or other differences in life history traits between morphs (Jonsson et al., 1988) that would affect shape.

Complex Interplay Between Benthic-Limnetic Traits and Secondary Sexual Traits

Despite both *cross* and *sex* being significant across ontogeny, these terms showed opposite tendencies (Fig. 3): while the effect of *sex* increased, *cross*, which had larger load than sex at month 18, greatly decreased towards month 36. We found that males were significantly larger than females at months 18 and 24 ($t = 3.134$, $p = 0.0018$ and $t = 2.29$, $p = 0.0013$ respectively), but no centroid size differences were found at month 36 ($t = -1.479$, $p = 0.14$; see SM7), suggesting that either the effect of size on the expression of sexual dimorphism is negligible or that it is dynamic, and its signal is diluted by other confounding factors.

We then asked whether ontogenetic trajectories between the two morphs differed and what the position of the hybrids was. Phenotypic trajectory analysis (PTA) showed overall differences in ontogenetic trajectories between each cross type (Fig. 5A). We found significant differences in trajectory location between all pairs ($p = 0.001$), direction (Pr angle $\leq$ 0.007 for each pair), and shape ($Pr>d = 0.001$ for each pair). However, only significant differences in the amount of shape change (i.e., absolute path trajectory lengths) were found between both intra-morph crosses and PLxSB ($Pr>d = 0.007$ and 0.004 respectively). Of the four cross types, PLxSB appeared to have the longest trajectory (maximum absolute path distance = 0.0672) (Fig. 5A). At month 36, cross type means converged in both PC1 and PC2, although morphological disparity at this time point increased, likely driven by -at least- individuals who reached sexual maturation and showed secondary sexual traits (SM8).

Since the onset of sexual maturation occurred during the experiment and sex had an important impact on shape (Fig. 2, SM9), ontogenetic trajectories for both sexes were additionally explored. PTA showed significant differences in trajectory location, directionality and shape between sexes ($p = 0.001$, $Pr > angle = 0.001$ and $Pr > d = 0.001$ respectively), although absolute path distances did not differ ($Pr > d = 0.94$) (Fig. 5B). At month 18, mean shapes of both sexes laid practically on the same point in the morphospace. However, at month 24, they diverged along PC2,

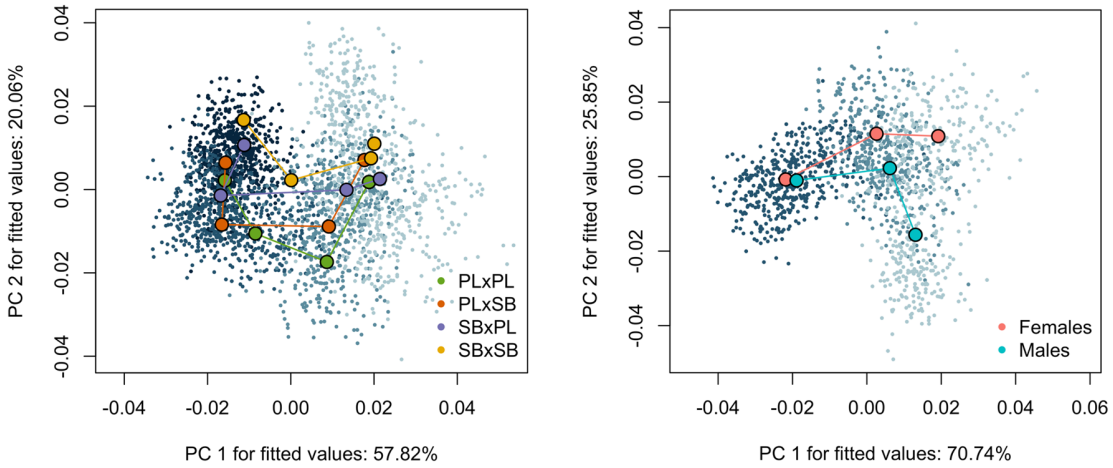

Fig. 5 Phenotypic Trajectory Analysis onto the first two principal components based on the covariance matrix of group means. Each point represents one individual. Descending color intensity depicts the different time points (i.e. (12), 18, 24 and 36). Large points represent mean shape estimates for each cross type (**A**) or sex (**B**) at each trajectory point, and are connected in chronological order

and differences increased markedly towards month 36, where the great morphological disparity seemed to be explained, to a larger degree, by sexual dimorphism. Additionally, morphological disparity in males was not only larger, but seemed to increase at a higher rate than morphological disparity in females (Table SM8, fig. SM8.1).

Discussion

In a long-running common garden experiment involving Arctic charr ecomorphs from lake Thingvallavatn (Iceland), we found that traits traditionally associated with benthic-limnetic adaptations have a genetic component. Interestingly, throughout ontogeny reciprocal hybrids between PL and SB showed intermediate, transgressive and parental-like phenotypes, indicating that the phenotypic outcome of hybrids is dynamic across time. Additionally, the onset of sexual maturation triggers differences in sex ontogenetic trajectories and shape variation at different time points. Taken together our results suggest that the interplay between traits associated with benthic-limnetic adaptations and traits associated with sexual dimorphism is complex and dynamic throughout ontogeny.

We asked whether differences in traits traditionally associated with benthic-limnetic adaptations were present in the different cross types. Geometric morphometric analyses revealed that traits associated to benthic-limnetic adaptations were detectable at each time point between SB and PL. These traits mostly comprise differences in the trophic apparatus, SB individuals having subterminal jaws, rounder snouts and deeper heads and bodies, whereas PLs were characterised for terminal jaws, pointed snouts and elongated heads and bodies. This result was in line with a recent study showing morphological differences in craniofacial morphology between PL and SB charr embryos reared in common garden conditions even before first feeding (Ponsioen, 2020). The genetic differences causing this phenotypic variation remain unknown and are likely to be complex. For instance, complex genetic architectures modulate distinct craniofacial structures in response to different selective pressures in benthic-limnetic cichlids from lake Malawi (Albertson & Kocher, 2005). Moreover, the functional differentiation between cichlids with suction vs. scrapping feeding apparatus is related to quantitative trait loci (QTL) ultimately influencing the shape of the articular and the dentary bones (Albertson et al., 2005; Parsons et al., 2011; Roberts et al., 2011). These QTLs, some having pleiotropic effect on the two bone structures, contain genes affecting bone development through different signalling pathways.

Patterns of shape variation in the reciprocal hybrids exhibited intermediate shapes at month 12, intermediate to transgressive shapes at month 18, and at month 24 most hybrids adopted a maternal phenotype. At month 36, major differences in shape variation did not occur between cross types, but between sexes. The different types of inheritance seen in the reciprocal hybrids indicates that the genetic component of the morphological traits unfolds in complex ways, and its expression, at least under the same environmental conditions, is asymmetrical and dependent on the parenthood. The hybrid's use of either benthic or limnetic resources may be effective, but not constant across ontogeny. The ontogenetic niche shift (see MM, study system) between PL and SB charr is believed to occur in juveniles (Sandlund et al., 1992). This means that selection against hybrids with intermediate phenotypes may occur at month 12, but might

be relaxed later in ontogeny, when hybrid phenotypic values tend towards the maternal morph. A recent study found that while the growth of hybrid embryos and early juveniles of PL and SB was similar to SB, covariance patterns between head shape and a set of traits including size was closer to PL's (Horta-Lacueva et al., 2021). Our long-running common garden experiment further indicates that this complexity persists throughout ontogeny.

The onset of sexual maturation plays a key role in explaining shape variation across ontogeny, especially in later stages. At month 36, extensive differences in shape were found between males and females, pointing towards strong genetic basis to sexual dimorphism. Members of the Salmonidae family are characterised by a marked sexual dimorphism, which is thought to be, to some extent, due to sexual selection (Fleming, 1996; Gaudemar, 1998). Classic examples of pronounced sexual traits in salmonids can be mostly found in males. These are often larger body sizes, pointier or even hooked snouts, exaggerated humps and bright colorations, which are thought to have arisen as a result of high male densities and competition. At month 36 males showed similar phenotypes to the ones outlined above, namely pointier snouts, larger heads and humped dorsal area. Moreover, the increase in morphological disparity of the males relative to the females may indicate that the development of secondary sexual traits in males is more heterogenous and that the mechanisms leading to it are complex (see e.g., Woram et al., 2003; Sutherland et al., 2019).

It is worth noting that the dynamic patterns of sexual dimorphism and traits associated with benthic-limnetic adaptations are reversed (i.e., the relative load of cross type decreased while the relative load of sex increased) alluding that the developmental programs driving sexual maturation and the processes driving benthic-limnetic morphological divergence are decoupled. Such view is further supported by both ecomorph and sex ontogenetic trajectories: mean shapes of different morphs converge at month 36, as morphological disparity dramatically increases, and sex ontogenetic trajectories diverge. Further evidence for decoupling of sexual maturation and benthic-limnetic morphological divergence can be found in the non-significant interaction between sex and cross (SM3), indicating that the cross type and sex independently affect shape.

With our data, we were not able to determine whether sexual dimorphism in the Thingvallavatn system has originated prior-, post-ecological diversification, or a combination of both. Considering Arctic charr colonised different water bodies in Iceland and that it parallelly evolved distinct ecomorphs (Gíslason et al., 1999; Jacobs et al., 2020; Kapralova et al., 2011; Snorrason & Skúlason, 2004), one can argue that what we observe is the result of ancestral sexual dimorphism and subsequent adaptive radiation (for other systems, see: Aguirre et al., 2008; Lisle & Rowe, 1803). This is further supported by a widespread strong sexual dimorphism within the salmonid clade (Fleming, 1996; Gaudemar, 1998). Alternatively, secondary sexual traits evolving after sympatric radiations should be exclusive within each ecomorph, unless assortative mate choice occurs before trait divergence starts (Bolnick & Doebeli, 2003; Doorn & Weissing, 2002; Parsons et al., 2011; Slatkin, 1984). In our study, sexes mainly differed in relative head size, body depth and snout shape, but no differences were observed in the shape of the jaws, usually associated with benthic-limnetic feeding. Although the overlap between observed differences in males and females and between ecomorphs is minor, some trait variations such as relative head size and body depth appear to be common. Such an association may potentiate ecological adaptation within morphs, in line with other studies showing complex relationships between processes of speciation and sexual dimorphism (Cooper et al., 2011; Parsons et al., 2011). This complexity of different morphological traits across ontogeny points towards a combination of ancestral origin of sexual dimorphism followed by reinforcement of secondary sexual traits after sympatric divergence.

Conclusions

We found that phenotypic traits associated with benthic-limnetic adaptations in PL, SB and their hybrids are present and are genetically controlled at different time points. Sexual maturation is key in this scenario, since developmental programs driving the onset of the breeding season override adaptive traits, in similar environments at certain time points. The interplay between traits associated with ecological diversification and sexual maturation during development is complex and more efforts should be directed towards studying their relationship in this and other adaptive radiation systems, emphasising its dynamism throughout ontogeny.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11692-022-09592-y.

Acknowledgements We thank Kári H. Árnason, Rakel Þorbjörnsdóttir, and Christian Beuvard for the maintenance of the experimental setup at the rearing facility at Verið, Sauðárkrókur (Hólar University College, Iceland).

Author Contributions MC conceptualised the study, conducted the analysis and wrote the manuscript. LP and QH collected the data, phenotyped the specimens and critically revised the manuscript. KHK conceived the study, established the crossing design, reared the embryos, collected the data and contributed to the writing of the manuscript. All authors gave their final approval for publication and agree to be accountable for the work therein.

Funding This work was fully funded by the Icelandic Centre of Research, RANNÍS (Icelandic Research Fund grant no. 1535–1533039 and 1535–1533090).

Data availability The data will be deposited onto the Dryad Digital Repository upon acceptance.

Declarations

Conflict of interest The authors declare no conflict of interests.

Ethical Approval The rearing and the experimental work was conducted in the facilities of Hólar University Aquaculture Research Station, which has an operational license under the Icelandic Aquaculture law (Law No. 71/2018). This law includes clauses of best practices for animal care and experimental work. 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.

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Supplementary material

Table S1. Crossing design with number of individuals and included in final analyses per family and time point.

cross	familiy	# ind			
		month 12	month 18	month 24	month 36
PLxPL	H1509	35	30	27	27
	H1513	35	30	26	25
	H1516	30	29	29	26
	H1517	30	-	-	-
	H1518	14	9	10	9
	H1525	-	52	37	35
	6	144	150	129	122
PLxSB	H1512	28	14	13	14
	H1514	23	20	22	22
	H1520	3	2	3	3
	H1524	29	29	27	28
	H1526	92	93	84	87
	H1527	54	48	41	43
	H1531	32	22	25	25
	7	261	228	215	222
SBxPL	H1505	24	17	16	15
	H1506	101	107	94	96
	H1507	72	76	76	72
	H1508	33	33	31	33
	4	230	233	217	216
SBXSB	H1502	4	16	16	15
	H1503	22	17	17	15
	2	26	33	33	30
	19				

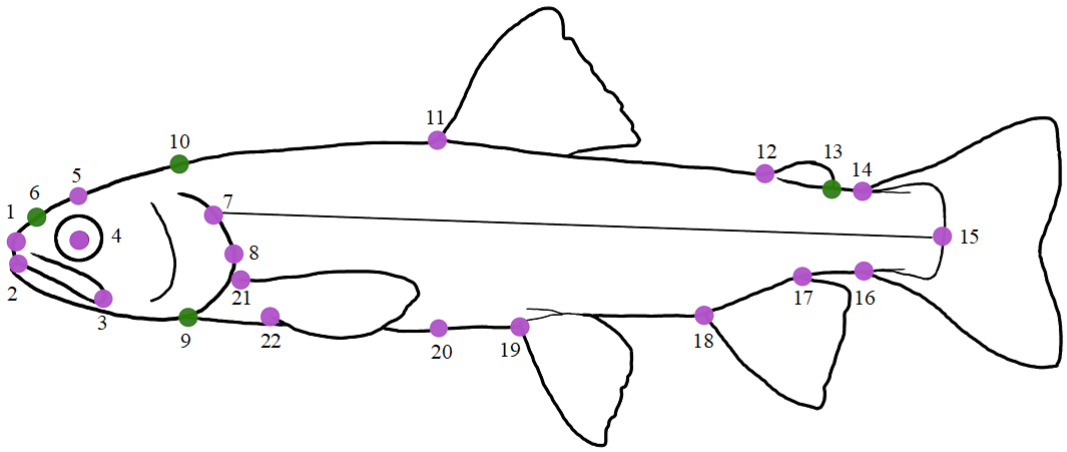


Fig. S2. Landmark (violet) and semilandmark (green) configuration used for geometric morphometrics analyses. Landmarks taken for scaling and unbending are not shown. Landmark description: L1, tip of the upper jaw; L2, tip of the lower jaw; L3, posterior tip of the maxila; L4, centre of the eye; L5, projection of L4 on the dorsal edge of the fish; SM6, middle point between L1 and L5; L7, beginning of the lateral line from the gill opening (also used for unbending); L8, most posterior point of the gill opening; SL9, ventral end of the gill opening; SL10, projection of SL9 on the dorsal edge of the fish; L11, anterior insertion of the dorsal fin; L12, anterior insertion of the adipose fin; SL13, posterior insertion of the adipose fin; L14, dorsal insertion of the caudal fin; L15, end of the spinal column; L16, ventral insertion of the caudal fin; L17, posterior insertion of the anal fin; L18, anterior insertion of the anal fin; L19, insertion of the pelvic fin; L20, projection of L11 on the ventral edge of the fish; L21, dorsal insertion of the pectoral fin; L22, ventral insertion of the pectoral fin.

Table S3.1. Significant terms affecting shape of sexed individuals phenotyped at 18, 24 and 36 months after fertilization in a mixed-model MANOVA using residual randomization. Error terms updated to account for nested families within cross types.

	d.f.	SS	MS	Rsq	F	Z	Pr (>F)
log(Csize)	1	0.078	0.0776	0.069	229.595	10.562	0.001 **
month	2	0.085	0.0427	0.076	126.196	12.387	0.001 **
sex	1	0.049	0.0489	0.044	144.58	9.644	0.001 **
cross	3	0.055	0.0183	0.049	4.061	4.484	0.001 **
cross:family	14	0.063	0.0045	0.056	13.352	17.552	0.001 **
log(Csize):month	2	0.009	0.0046	0.008	13.609	7.748	0.001 **
log(Csize):sex	1	0.025	0.0246	0.022	72.617	9.004	0.001 **
log(Csize):month:sex	2	0.010	0.0049	0.009	14.612	8.224	0.001 **
log(Csize):month:sex :cross:family	99	0.103	0.0010	0.092	3.086	18.015	0.001 **
Residuals	1231	0.416	0.0003	0.371			
Total	1356	1.121					

Table S3.2. Final mixed-model MANOVA using residual randomization on shape of the full dataset of individuals phenotyped at 12, 18, 24 and 36 months after hatching. Error terms updated to account for nested families within cross types.

	d.f.	SS	MS	Rsq	F	Z	Pr (>F)	
log(Csize)	1	0.528	0.5275	0.262	1403.14	12.60	0.0010	**
month	3	0.257	0.0855	0.128	227.52	16.02	0.0010	**
cross	3	0.090	0.0299	0.045	4.22	4.30	0.0010	**
log(Csize):month	3	0.022	0.0074	0.011	19.71	10.42	0.0010	**
log(Csize):cross	3	0.017	0.0056	0.008	0.78	-0.71	0.7560	
cross:family	14	0.099	0.0071	0.049	18.83	18.02	0.0010	**
log(Csize):month: cross:family	64	0.109	0.0017	0.054	4.53	18.84	0.0010	**
Residuals	2367	0.890	0.0004	0.443				
Total	2458	2.011						

SM 4. Pairwise comparisons in means and variances between cross types at each time point, using 1000 permutations.

Table S4.1. Month 12 – Mean pairwise differences by cross type.

Pairwise distances between means, plus statistics				
	d	UCL (95%)	Z	Pr>d
PLxPL : PLxSB	0.0093	0.0047	7.493	0.001
PLxPL : SBxPL	0.0145	0.0048	13.191	0.001
PLxPL : SBxSB	0.0214	0.0115	7.017	0.001
PLxSB : SBxPL	0.0096	0.0033	12.815	0.001
PLxSB : SBxSB	0.0174	0.0109	5.456	0.001
SBxPL : SBxSB	0.0101	0.0111	1.191	0.113

Table S4.2. Month 12 – Observed variances by cross type.

Observed variances by group	
PLxPL	0.000251
PLxSB	0.000278
SBxPL	0.000273
SBxSB	0.000238

Table S4.3. Month 12 – Variance pairwise differences by cross type.

Pairwise distances between variances, plus statistics				
	d	UCL (95%)	Z	Pr>d
PLxPL : PLxSB	2.74E-05	3.055E-05	1.581	0.088
PLxPL : SBxPL	2.23E-05	3.103E-05	1.056	0.165
PLxPL : SBxSB	1.25E-05	5.938E-05	-0.636	0.691
PLxSB : SBxPL	5.12E-06	2.476E-05	-0.639	0.673
PLxSB : SBxSB	3.99E-05	5.744E-05	0.973	0.159
SBxPL : SBxSB	3.48E-05	5.664E-05	0.650	0.23

Table S4.4. Month 18 - Mean pairwise differences by cross type.

Pairwise distances between means, plus statistics				
	d	UCL(95%)	Z	Pr>d
PLxPL : PLxSB	0.0140	0.0039	15.4466	0.001
PLxPL : SBxPL	0.0179	0.0037	20.2788	0.001
PLxPL : SBxSB	0.0253	0.0077	13.7834	0.001
PLxSB : SBxPL	0.0101	0.0037	11.6984	0.001
PLxSB : SBxSB	0.0203	0.0079	10.6856	0.001
SBxPL : SBxSB	0.0174	0.0078	9.0448	0.001

Table S4.5. Month 18 – Observed variances by cross type.

Observed variances by group	
PLxPL	0.000251
PLxSB	0.000257
SBxPL	0.000279
SBxSB	0.000236

Table S4.6. Month 18 – Variance pairwise differences by cross type

Pairwise distances between variances, plus statistics				
	d	UCL(95%)	Z	Pr>d
PLxPL : PLxSB	6.37E-06	2.87E-05	-0.556	0.644
PLxPL : SBxPL	2.82E-05	2.78E-05	1.929	0.043
PLxPL : SBxSB	1.49E-05	5.11E-05	-0.392	0.575
PLxSB : SBxPL	2.19E-05	2.41E-05	1.548	0.082
PLxSB : SBxSB	2.12E-05	4.96E-05	0.079	0.399
SBxPL : SBxSB	4.31E-05	4.99E-05	1.515	0.089

Table S4.7. Month 24 - Mean pairwise differences by cross type.

Pairwise distances between means, plus statistics				
	d	UCL (95%)	Z	Pr>d
PLxPL : PLxSB	0.0120	0.0042	11.4171	0.001
PLxPL : SBxPL	0.0212	0.0039	20.3373	0.001
PLxPL : SBxSB	0.0302	0.0076	15.8586	0.001
PLxSB : SBxPL	0.0153	0.0037	17.1499	0.001
PLxSB : SBxSB	0.0219	0.0074	12.1959	0.001
SBxPL : SBxSB	0.0151	0.0072	7.6740	0.001

Table S4.8. Month 24 – Observed variances by cross type.

Observed variances by group	
PLxPL	0.000370
PLxSB	0.000307
SBxPL	0.000241
SBxSB	0.000322

Table S4.9. Month 24 – Variance pairwise differences by cross type.

Pairwise distances between variances, plus statistics				
	d	UCL (95%)	Z	Pr>d
PLxPL : PLxSB	6.35E-05	3.78E-05	4.121	0.001
PLxPL : SBxPL	1.29E-04	3.92E-05	8.890	0.001
PLxPL : SBxSB	4.78E-05	7.20E-05	0.923	0.166
PLxSB : SBxPL	6.52E-05	3.54E-05	4.723	0.001
PLxSB : SBxSB	1.57E-05	6.92E-05	-0.553	0.641
SBxPL : SBxSB	8.09E-05	6.82E-05	2.600	0.020

Table S4.10. Month 36 - Mean pairwise differences by cross type.

Pairwise distances between means, plus statistics				
	d	UCL (95%)	Z	Pr>d
PLxPL : PLxSB	0.0100	0.0056	6.0334	0.001
PLxPL : SBxPL	0.0104	0.0055	6.6022	0.001
PLxPL : SBxSB	0.0166	0.0109	4.5607	0.002
PLxSB : SBxPL	0.0105	0.0044	9.1559	0.001
PLxSB : SBxSB	0.0121	0.0105	2.7498	0.018
SBxPL : SBxSB	0.0124	0.0104	3.0227	0.010

Table S4.11. Month 36 – Observed variances by cross type.

Observed variances by group	
PLxPL	0.000583
PLxSB	0.000614
SBxPL	0.000538
SBxSB	0.000548

Table S4.12. Month 36 – Variance pairwise differences by cross type.

Pairwise distances between variances, plus statistics				
	d	UCL (95%)	Z	Pr>d
PLxPL : PLxSB	3.15E-05	7.84E-05	-0.013	0.443
PLxPL : SBxPL	4.47E-05	7.68E-05	0.625	0.244
PLxPL : SBxSB	3.44E-05	1.45E-04	-0.531	0.64
PLxSB : SBxPL	7.62E-05	6.34E-05	2.552	0.017
PLxSB : SBxSB	6.59E-05	1.38E-04	0.233	0.346
SBxPL : SBxSB	1.03E-05	1.38E-04	-1.054	0.873

Figure S6. Two first principal components of month 36, showing that either pure crosses or reciprocal hybrids did not cluster towards the "most male or female" area of the morphospace. Each point represents one individual and shaded areas depict 95% confidence ellipses.

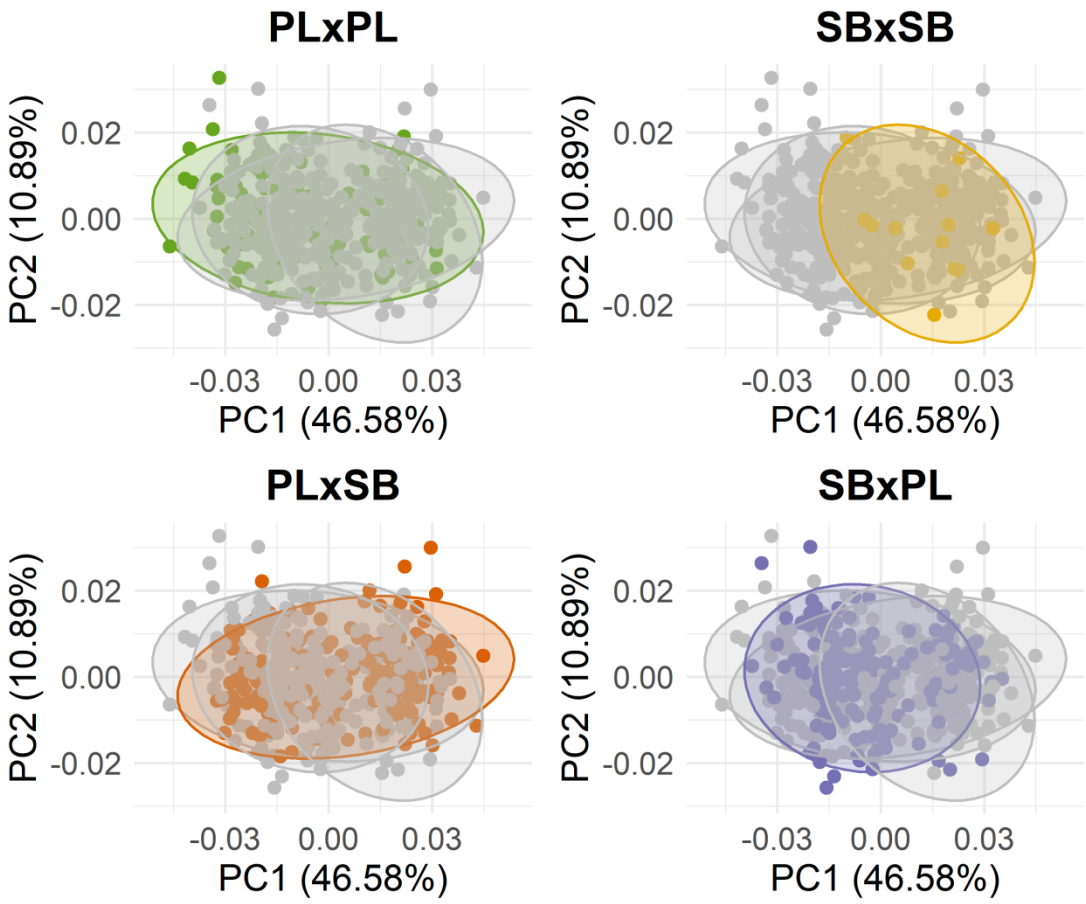


Table S7.1. Centroid size summary by *month:cross*.

month	cross	Csize mean (cm)	std. dev (cm)
12	PLxPL	8.94	± 0.94
	PLxSB	8.86	± 0.67
	SBxPL	9.33	± 0.84
	SBxSB	9.35	± 0.84
18	PLxPL	13.15	± 1.49
	PLxSB	11.82	± 1.47
	SBxPL	11.88	± 1.47
	SBxSB	13.71	± 1.84
24	PLxPL	18.81	± 2.45
	PLxSB	18.52	± 2.16
	SBxPL	18.58	± 2.13
	SBxSB	19.62	± 2.72
36	PLxPL	25.46	± 4.75
	PLxSB	24.90	± 4.53
	SBxPL	25.57	± 3.31
	SBxSB	23.35	± 3.50

Table S7.2. Centroid size summary by *month:sex*.

month	sex	Csize mean (cm)	std. dev (cm)
12	NA	9.07	± 0.82
18	F	12.08	± 1.58
	M	12.57	± 1.68
	NA	11.95	± 1.51
24	F	18.7	± 2.19
	M	19.19	± 2.05
	NA	17.69	± 2.39
36	F	25.87	± 3.58
	M	25.41	± 4.29
	NA	23.83	± 4.30

Table S8.1. Morphological disparity from Phenotypic Trajectory Analysis per sex and cross type at different time points.

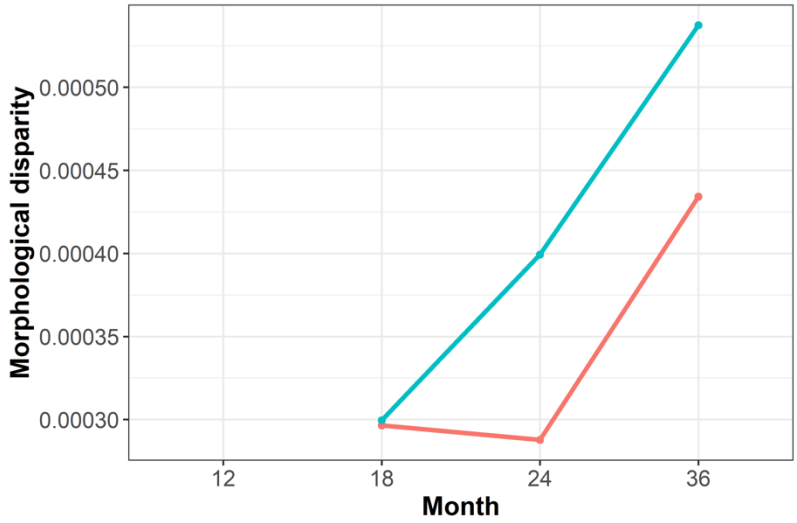
time point (month)	morphological disparity within time point	sex	morphological disparity within month:sex	cross type	morphological disparity within month:cross
12	2.747×10^{-4}	f	-	PLxPL	2.507×10^{-4}
				PLxSB	2.781×10^{-4}
		m	-	SBxPL	2.730×10^{-4}
				SBxSB	2.382×10^{-4}
18	2.722×10^{-4}	f	2.965×10^{-4}	PLxPL	3.240×10^{-4}
				PLxSB	3.091×10^{-4}
		m	2.995×10^{-4}	SBxPL	3.212×10^{-4}
				SBxSB	3.002×10^{-4}
24	3.179×10^{-4}	f	2.878×10^{-4}	PLxPL	3.700×10^{-4}
				PLxSB	3.066×10^{-4}
		m	3.993×10^{-4}	SBxPL	2.414×10^{-4}
				SBxSB	3.222×10^{-4}
36	5.974×10^{-4}	f	4.342×10^{-4}	PLxPL	5.830×10^{-4}
				PLxSB	6.136×10^{-4}
		m	5.374×10^{-4}	SBxPL	5.377×10^{-4}
				SBxSB	5.488×10^{-4}

S8.2. Covariates effects from independent MANOVAs at for months 12, 18, 24 and 36, using residual randomization. Error terms updated to account for nested families within cross types.

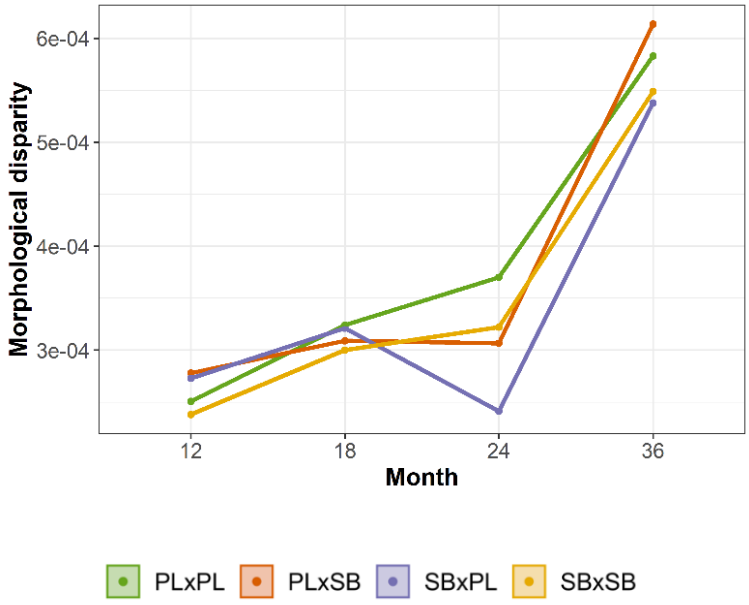
	Month 12				**Month 18**			
	Rsq	Z	pr (>F)		Rsq	Z	pr (>F)	
log(Csize)	0.037	8.136	0.001	**	0.080	10.191	0.001	**
cross	0.106	4.374	0.001	**	0.131	5.310	0.001	**
cross:family	0.110	13.774	0.001	**	0.097	14.776	0.001	**
log(Csize):cross	0.020	-0.831	0.788		0.023	0.360	0.037	
log(Csize):cross:family	0.016	0.596	0.283		0.016	1.872	0.036	*
Residuals	0.675				0.542			

	Month 24				**Month 36**			
	Rsq	Z	pr (>F)		Rsq	Z	pr (>F)	
log(Csize)	0.042	8.229	0.001	**	0.082	7.091	0.001	**
cross	0.197	4.737	0.001	**	0.044	2.618	0.008	**
cross:family	0.006	14.375	0.001	**	0.071	7.495	0.001	**
log(Csize):cross	0.139	-4.823	1.000		0.009	-1.241	0.899	
log(Csize):cross:family	0.023	3.331	0.002	**	0.037	4.172	0.001	**
Residuals	0.574				0.727			

S8.3. Morphological disparity across time in males (blue) and females (red).



S8.7. Morphological disparity across time in the different morphs.



PAPER II

The genomic architecture of benthic-limnetic ecological differentiation

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Abstract

Knowing the genomic basis of the morphological changes involved in resource polymorphisms is key to understand ecological specialisation and ultimately, ecological adaptation. The Arctic charr from lake Thingvallavatn (Iceland) has diverged in sympatry along a benthic-limnetic ecological axis, resulting in four morphs (two benthic, a small and a large benthic, and two limnetic, a planktivorous and a piscivorous). These ecomorphs have unique morphological characteristics which may reflect adaptations to living in benthic and limnetic environments. In this study, we investigated the genetic architecture of phenotypic traits associated with specialisations to benthic and limnetic habitats using the Arctic charr from Thingvallavatn as a model. For that, we generated a high-resolution linkage map from two Arctic charr morphs (the small benthic and the planktivorous) and conducted QTL (quantitative trait loci) mapping analyses to characterize the genomic basis of variation in ecological traits that differ between ecomorphs. We found that many of the explored morphological traits have a genetic basis, and were predominantly explained by single QTL with moderate to high percentages of PVE (phenotypic variance explained). These QTL were sparsely distributed across the genome, with few instances of colocalization. The most consistent QTL detected across families and/or genomic region explained shape differences related to the relative size of the head, maxilla shape, and peduncle depth. Our findings underscore the pivotal role of genetics in morphological divergence and shed light on the genomic mechanisms driving ecological specialization in sympatric Arctic charr ecomorphs.

Keywords: genetic architecture, QTL mapping, linkage map, Arctic charr, benthic-limnetic

Introduction

Exploring the genomic architecture of traits related to ecological specialisations is crucial for understanding how organisms evolve and thrive in diverse environments. Complex genetic architectures often drive phenotypic differences among populations, however it may be challenging to disentangle the genomic regions responsible for traits related to different evolutionary processes, such as specialisation, adaptation or speciation. Adaptive radiations where individuals have undergone ecological specialisation offer unique opportunities to address questions related to how diversity is generated in short periods of time (McGee et al., 2020; Naciri & Linder, 2020; Schluter, 2000; Seehausen, 2006). Iconic examples of adaptive radiations include Darwin's finches (Bowman, 1961; Grant, 1999; Grant & Grant 2007; Lack, 1945), African Rift lake cichlids (Cooper et al., 2010; Fryer, 1972), Hawaiian spiders (Gillespie, 2004), Hawaiian honeycreepers (Amadon, 1950; Freed et al., 1987), Caribbean Anolis lizards (Losos, 2011) or sticklebacks (Bell & Foster, 1994). Common denominators in such radiations are sufficient genetic variation and an environment that presents an ecological opportunity (Hendry & Kinnison, 2001; Losos & Mahler, 2010; Wellborn & Langerhans, 2015).

Most phenotypic traits associated with ecological diversification are often explained by complex genomic architectures, usually involving multiple loci across the genome (i.e., quantitative trait loci, or QTL) (Franchini et al., 2014; Stankowski et al., 2023), although examples of simpler genetic architectures exist (Barría et al., 2021; Barson et al., 2015; Chan et al., 2010; Colosimo et al., 2004; Cresko et al., 2004). One of the main biological questions in the context of ecological diversification is whether phenotypic specialisations result from a few QTL of large effects (e.g. (Martin et al., 2017), many QTL of small effects (e.g.,(Henning et al., 2017)) or a combination of both (Coughlan et al., 2021; Gerwin et al., 2021). There are two evolutionary hypotheses behind such patterns: (1) the "island hypothesis", where a few QTL of large effects arise early in the divergence history, promoting rapid reproductive isolation and making selection more effective (e.g., islands of speciation in cichlids and sticklebacks (Malinsky et al., 2015; Marques et al., 2016), or (2) the "genome-wide" hypothesis, where ecological differentiation occurs through the accumulation of many small-effect mutations distributed throughout the genome (i.e., polygenic architectures) that can be as effective to create a robust adaptive response even in the presence of geneflow (Henning et al., 2017; Kautt et al., 2020; Nosil et al., 2017).

Three main methodological approaches are being used to test these hypotheses. The first, most traditional one, is conducting QTL mapping using laboratory-generated crosses. This method takes advantage of known genetic lines and controlled conditions, though breeding non-model organisms in captivity can be resource-intensive. Moreover, designing a family-based study requires careful selection of crosses, as these choices impact both the power to detect QTL and the complexity of the analyses (reviewed by (Ashton et al., 2017)). The second methodological approach consists of conducting QTL mapping on wild pedigrees, but this approach is rarely used due to the lack of suitable systems and sampling challenges (Ashraf et al., 2022; Bérénos et al., 2014; Slate, 2005). More modern approaches, such as Genome-Wide Association Studies (GWAS) have facilitated the detection of multiple genomic regions putatively linked to ecological diversification, but they suffer from non-causative phenotype-genotype associations, limited power and a substantial number of false positives (Gienapp et al., 2017; Hansson et al., 2018; Santure & Garant, 2018).

Even though QTL mapping has been criticized for its lack of precision, there has been a recent resurgence of this approach in ecological and evolutionary research. This resurgence is primarily due to a growing interest in exploring the nature of polygenic (or ”omnigenic” (Mathieson, 2021) complex traits, often in an ecological framework, to unravel the genetic basis of traits related to adaptation and reproductive isolation (Albertson et al., 2014; Feller et al., 2020; Feulner et al., 2018; Gerwin et al., 2021; Henning et al., 2014; Kudo et al., 2015). This renewed interest is further supported by technical advancements, including affordable high-resolution genomic sequencing, improved statistical techniques, and the integration with either genomic approaches such as selection scans, GWAS or with fitness experiments (Hansson et al., 2018; Venu et al., 2024). An ideal system to study the genomic architecture behind ecological differentiation is a geographically isolated ecosystem, inhabited by a metapopulation which has diverged into one or more reproductively isolated populations. Ideally these populations should be characterised by high levels of phenotypic and genotypic variability while having a relatively simple and well-documented ecology. Northern freshwater fish, particularly salmonids, serve as ideal systems for such studies due to their relatively young and simple evolutionary history, coupled with a remarkable intra- and inter-specific variation (Klemetsen, 2013; Salisbury & Ruzzante, 2022). This variation has occasionally led to rapid ecological diversification (Klemetsen, 2013; Peichel et al., 2001; Snorrason & Skúlason, 2004). Additionally, salmonid systems are highly advantageous to study due to the availability of genetic and computational resources. This abundance of resources is largely attributed to their economic and social significance (Houston & Macqueen, 2019; Yáñez et al., 2014) and the interest in investigating whole genome duplication (WGD) events and their consequences in these species (Allendorf et al., 1984; Berthelot et al., 2014; Glasauer & Neuhauss, 2014; Macqueen & Johnston, 2014; Ouellet-Fagg, 2023). As a result, a large body of work exists trying to establish the genomic architecture of various quantitative traits in salmonid fish (Christensen et al., 2018; Gharbi et al., 2006; Guyomard et al., 2012; Kodama et al., 2014; Küttner et al., 2011; Leitwein et al., 2017; Ouellet-Fagg, 2023; Sakamoto et al., 2000). Among the most studied traits are body weight and condition factor (e.g., (Gutierrez et al., 2012; Reid et al., 2005; Sauvage et al., 2012)), but also other life history traits, such as size and age at maturity (Barson et al., 2015) or susceptibility to diseases such as the infectious pancreatic necrosis (IPN) (Houston et al., 2010).

The Arctic charr (*Salvelinus alpinus*) from lake Thingvallavatn, Iceland is an excellent system to study the genomic architecture behind ecological diversification. Thingvallavatn is a large (Iceland largest, of 83km^2), deep (maximum depth = 114m) and oligotrophic lake (Sandlund et al., 1992) with multiple discrete habitats which offer a myriad of ecological resources (i.e., ecological opportunities) (Snorrason & Skúlason, 2004). There is a large pelagic zone with a high phytoplankton production and a benthic zone, the later subdivided in three main areas: (1) the stony littoral zone (0-10m deep), characterised by a hard bottom with scattered stony substrate, porous lava stones and cervices, allowing a highly diverse habitat for the zoobenthos, (2) the *Nitella* zone (10-25m deep), a densely vegetated area of the green algae *Nitella opaca,* with abundance of three-spined sticklebacks, and (3) the profundal zone (> 25m deep), formed by a soft bottom with diatomic gyttja substrate (Sandlund et al., 1992). The lake was colonised once by a single population of Arctic charr at the end of the last glacial period and has since then diverged along the classic benthic-limnetic ecological axis via resource polymorphism (i.e., specialising in exploiting the resources present in the zones described above) (Malmquist et al., 1992; Sandlund et al., 1992; Snorrason et al., 1994). There are two benthic charr populations, a small and a

large benthic charr (SB and LB) and two limnetic charr populations, a small planktivorous and a large piscivorous (PL and PI). The two benthic morphs feed on macroinvertebrates on the lava bottom of the lake, while the limnetic morphs feed on zooplankton in the pelagic zone (in the case of the PL charr) or on three-spined sticklebacks in the *Nitella* zone (in the case of the PI charr). To facilitate access to different prey types in benthic and limnetic habitats, a series of phenotypic changes have occurred in these morphs over time.

Morphologically, the benthic morphs are characterised by larger heads, deeper bodies and subterminal jaws and rounder snouts. Such morphological characteristics are thought to facilitate the collection of macroinvertebrates from the benthos. Additionally, benthic fish have thicker pectoral fins which are thought to help their maneuverability within the interstices of the lava bottom. The limnetic morphs (PI and PL charr) have smaller heads relative to their slender bodies, pointy snouts with terminal jaws, traits which are thought to improve feeding in a water column (i.e. help with catching floating or swimming preys) (Jonsson et al., 1988; Malmquist et al., 1992; Sandlund et al., 1987; Skúlason et al., 1993; Snorrason et al., 1989). Backwards simulations have pointed towards the benthic and the limnetic branches diverging early in the population history from an anadromous ancestor, with this divergence likely being boosted by a short micro-allopatry period (Brachmann et al., 2022; Kapralova et al., 2011; de la Cámara et al., *in prep*). Secondary colonisations or species introductions are, to date, non-existent, likely due to the complex geological history of the lake (Ingólfsson et al., 1995).

A recent effort to disentangle the genomic architecture behind morphological changes involved in Arctic charr ecological diversification in Iceland was conducted by (Ouellet-Fagg, 2023) using QTL mapping. This study is the first to examine the developmental architecture of morphological traits in Arctic charr, focusing on the effects of ontogeny (age) and environment (diet). Multiple families originating from both Icelandic and North American populations were used to generate new Arctic charr linkage maps, combining microsatellites, sex-determining genes and SNPs. Ouellet (2023) performed QTL mapping on eight F1 full-sib progenies from two Icelandic lakes, including Thingvallavatn (intra-crosses of the PL and LB morphs). The fish were exposed to two different diet treatments to explore the combined effects of genetics and plasticity on shape variation. Additionally, the fish were sampled at two developmental stages to look at the allometric relationships of ontogenic growth on shape. Highly polygenic architectures for body shape and size were reported in this study, with few cases of QTL co-localisation, one of them on linkage group AC11, which appeared to explain variation in different of traits (i.e., jaw length, eye diameter, body depth, caudal fin depth and body size). Previously, a less dense Icelandic Arctic charr linkage map using only aquaculture lines was employed to perform QTL mapping on body weight, condition factor, and age of sexual maturation, traits significant to both aquaculture practices and ecological studies (Küttner et al., 2011). Briefly, both studies searched for the genetic architecture of morphological variation within morphs, which was found to be polygenic, with QTL accounting for moderate to high effects. The present study on the other hand, focusses on the genetic architecture behind morphological variation seen between morphs, which likely plays a role in the Arctic charr ecological divergence in Thingvallavatn. This represents a major leap forward in understanding the ecological and evolutionary processes shaping the system. Furthermore, other aspects of our crossing design were crucial for expanding on previous studies. First, using the two small morphs as parental lines (PL and SB charr) allowed us to focus solely on benthic-limnetic specialisations by eliminating discrete body size differences. Second, inter-

crossing the parental lines to the second generation generated a variety of cross types (i.e., F2 and BC1), increasing the diversity of recombination events. Third, conducting our experiment in common garden conditions excluded environmental effects.

In this study we aimed to construct a dense, high-quality linkage map and to conduct QTL mapping using an array of quantitative traits putatively associated with ecological differentiation across benthic and limnetic habitats. For this, we focused on the two smallest morphs of Arctic charr from Thingvallavatn, the SB- and the PL-charr, because they represent two genetically distinct and homogeneous groups and have evolved along the benthic-limnetic ecological axis of divergence (Brachmann et al., 2022; de la Cámara et al., *in prep*). Given the evolutionary histories of the morphs, we asked (1) whether the benthic-limnetic traits were explained by a few QTL of large effects, multiple QTL of small effects or a combination of both, and (2) how these QTL will be distributed across the genome (i.e., whether the QTL will be homogeneously distributed across the LGs, or whether some LGs will harbour a larger number of QTL than others). Additionally, we asked which phenotypic traits were consistently explained by QTL across cross types, families, effect sizes and chromosomal regions, and how they are related to adaptations to benthic and limnetic habitats.

Material and methods

Sampling and morph selection

Wild Arctic charr specimens were caught in lake Thingvallavatn in the beginning of October 2015, using nets of mesh sizes ranging from 10 to 25mm. Sexually mature fish were classified to morph following (Snorrason et al., 1989). The planktivorous (PL) and small benthic (SB) charr were selected as the parental lines for the QTL experiment. The reasoning behind the selection of these two morphs is multi-fold. First, (1) they have diverged along the benthic-limnetic ecological axis via resource polymorphism (Malmquist et al., 1992; Sandlund et al., 1992). This means that benthic-limnetic morphological traits will likely reflect morphological adaptations to both niches. Second (2), the SB and PL morphs are morphologically distinguishable as adults (Malmquist et al., 1992; Snorrason et al., 1994; Snorrason et al., 1989), and no genetic identification was needed prior to breeding. Furthermore (3), the SB and PL charr are more genetically homogeneous compared to the complex patterns of introgression seen in LB and PI morphs (de la Cámara et al., *in prep.*). Despite the fact that the generation time in salmonids is considered long compared to other species commonly used in QTL experiments (Ashton et al., 2017), PL and SB have shorter generation times (4) compared to LB and PI, and overlapping spawning times (5) (Jonsson et al., 1988; Skúlason et al., 1989) facilitating the breeding and rearing of the families.

Crossing design

Intra-morph and hybrid F1 embryos were reared in common garden conditions as described in de la Cámara et al., 2023 in the facilities of Hólar University Aquaculture Research Station. Rearing F1 hybrids from PL and SB charr from Thingvallavatn is possible in captivity (e.g., (de la Cámara et al., 2023; Horta-Lacueva et al., 2021), even though they rarely interbreed in the wild (Brachmann et al., 2021). In 2018 a few F1 fish had reached sexual maturation, which allowed to make one F2 cross (H18_05, **Table 1**). By 2019 the majority of the fish had reached sexual maturation, which allowed to make the remaining of the crosses (**Table 1**). F1 reciprocal hybrids were either crossed amongst themselves to generate intercrosses (i.e., F2 crosses) or with the intra-morph crosses to generate backcrosses (i.e., BC1 crosses) (**Table 1**). A total of six Arctic charr families were generated between 2018 and 2019, with 454 individuals (**Table 1**) and were reared in common garden conditions at the facilities of Hólar University Aquaculture Research Station. Mortality throughout the experimental setup for the F1 generation was low (less than 10%), and higher levels of mortality were not attributed to any specific cross type or family.

Table 1. Families used for the QTL experiment. The family ID indicates the year when the cross was made (H18 in 2018 and H19 in 2019). The cross type indicates whether the cross was an intercross (F2) or a backcross (BC1). The cross order in the columns „Female parent“ and „Male parent“ indicates the husbandry (i.e.: FEMALE x MALE) and N individuals is the number of individuals in each family.

Family ID	Cross type	Female parent	Male parent	N individuals
H18_05	F2	PL x SB	PL x SB	51
H19_101	BC1	PL x SB	SB x SB	42
H19_103	BC1	PL x SB	SB x SB	105
H19_106	BC1	PL x SB	PL x PL	17
H19_109	F2	SB x PL	SB x PL	146
H19_111	BC1	PL x SB	SB x SB	93

Phenotyping and generation of the phenotypic matrix

Collection of phenotypic data

The grandparents (F0) were photographed before the crossing experiment began. The parents (F1) were photographed at 12-, 18-, 24- and 36-months post-fertilisation. Morphological analyses were conducted on a larger F1 data set (≈600 individuals per time point, (de la Cámara et al., 2023) to determine the timing of maximum morphological divergence in ecologically relevant traits related to benthic-limnetic axes of divergence. We found that the maximum morphological divergence can be detected at 18 months post-fertilisation, corresponding to a time before any secondary sexual traits become relevant to the overall shape of the fish. BC1 and F2 individuals were then photographed at 18 months post-fertilisation, except for family H1805 (photographed at 14 months post-fertilisation, because the optimum timing for phenotyping was not known at that time. Photos were taken on the charr's left lateral side with a fixed digital camera (Canon EOS 650D and 100 mm macro lens) along with a ruler for scaling.

Geometric morphometrics analyses

We placed 29 landmarks on each photo using Stereomoprh (Olsen & Westneat, 2015) (**SM - Fig. 1**), following (Adams & Huntingford, 2004; de la Cámara et al., 2023; Parsons et al., 2010). For scaling, two additional landmarks were placed on a ruler and were removed before performing Procrustes superimposition. For each individual fish the centroid size (the square root of the sum of squared distances of the landmarks to the centroid) was extracted during Procrustes superimposition and used for downstream corrections for body size (de la Cámara et al., 2023). We corrected for potential bending effects by placing 5 equidistant landmarks along the lateral line of each fish (a-d, 25 in **SM – Fig. 1**) and implementing the unbending tool in tpsUtil (Rohlf, 2015). Landmarking of the data was conducted by two people. To ensure the robustness of the data, thirty random individuals from different cross types and families were landmarked three times by each person, obtaining a high repeatability within and between ($p < 0.05$). Analysis of shape was performed using the geometric morphometrics package *geomorph* v4.0.0 and *RRPP* v1.0.0 (Adams et al., 2024; Baken et al., 2021; Collyer & Adams, 2024; Collyer & Adams, 2018) and following (de la Cámara et al., 2023).

Linear measurements

Linear measurements were extracted by calculating the interlandmark distances from the landmark configuration described above. Linear measurements were targeted to capture major shape differences associated to benthic and limnetic ecologies in Arctic charr (Adams & Huntingford, 2004; de la Cámara et al., 2023; Franklin et al., 2018; Jónsdóttir et al., 2024; Skúlason et al., 1989). As a result, thirteen traits were selected for further QTL mapping. These were: eye diameter (ED), snout to eye (Sn_E), head length (HL), maxilla depth (MD), maxilla length (ML), distance from the lower jaw to end of the maxilla (Lj_Em), head depth1 (HD1), head_depth2 (HD2), body depth (BD), and depth of the pectoral (PF), anal (AnF), adipose (AdF) and dorsal fin (DF) (**Fig. 2a)**. Body size correction was done for all studied traits by dividing each trait by the centroid size.

Normality tests were performed on each trait and family using Saphiro-Wilk tests. We complemented these tests with density and Q-Q plots for each trait and family. The detection of outliers was conducted by calculating the Z-scores for each trait within families. Individuals were considered outliers for a trait when the z-score was < -3.29 or > 3.29.

Correlation tests

We performed correlation tests among the size measurements (*i.e.*, Csize, weight and length), the 13 linear measurements mentioned above, the first 10 Principal Component scores (PC 1-10) and the first 10 residuals of those PC scores against Csize (V 1-10). Correlation tests were conducted to (1) explore the morphological relationships among the chosen (or targeted) linear measurements and (2) to understand if and how the PC scores capture the traits explained solely by linear measurements. Correlations were calculated with the *correlation* function in the r/correlation package (Makowski et al., 2020) and plotted using the *corrplot* R package (Wei et al., 2021) and custom scripts.

Genotyping and generation of the genotypic matrix

DNA extraction, construction and sequencing of the ddRAD libraries

DNA extraction was conducted on muscle or fin tissue using a phenol-chloroform protocol based on (Taggart et al., 1992). A custom ddRAD sequencing library preparation protocol was conducted following (Lagunas et al., 2023). A total of 454 samples distributed in four libraries (one library per lane) were sequenced with HiSeq X-ten at BGI HongKong.

Quality control, genotype calling and loci filtering

Raw reads were demultiplexed using *process radtags* in Stacks v.2.62 (Catchen et al., 2013; Rochette et al., 2019) and reads were truncated to 115bp. Reads with uncalled bases or with a lower phred score than 33 were removed. The resulting reads were aligned to the *Salvelinus alpinus* (based on the LB charr from Thingvallavatn) reference genome (*unpublished*) with the bwa-mem2 algorithm. *samtools* (Danecek et al., 2021) was used to generate and sort the individual‘s bamfiles, and the Stacks module *gstacks* for SNP calling. The *populations* module in Stacks was used to select the first SNP per rad-tag and those loci present in a minimum of three populations with a maximum observed heterozygosity of 66%.

Sex determination of the progeny

As sex has a significant effect on shape throughout ontogeny (de la Cámara et al., 2023), we needed to account for sex when conducting the QTL models. We did that by adding sex as a covariate. At the time of phenotypic data collection (i.e., 14 and 18 months after fertilisation) it was not possible to visually determine the sex of the fish. Thus sex was determined by amplifying with PCR the *SdY* gene with the primer combinations SdY-F2 and SdY-R4 or SdY-F3 and SdY-R5 (**SM – Table 1**). The SdY gene would only amplify in males (Yano et al., 2013). For amplification control, we used the ETBR2 gene (**SM – Table 1**).

Linkage map construction

Linkage map construction of one consensus male map and one consensus female map across families was performed using LepMap3 (Rastas, 2017). Only individuals that passed the quality requirements during filtering (see *Quality control, genotype calling and loci filtering*) were used. First, each specimen's pedigree was confirmed using the LepMap3 IBD module. Then, the pipeline was run with the options: *ParentCall2* with *halfsibs=1* given that families H19_103 and H19_109 have the same grandfather (*i.e.,* 1502-10_fin_1). This step resulted in a total of 843645 markers, of which 53853 were informative (6.38%). With the *Filtering2* module, markers that were non-informative, with more than 50% missing data, with minor allele frequency of less than 5% and with moderate segregation distortion ($\chi 2$ test, $p < 0.001$) were excluded. After filtering, 4257 markers were to be assigned into different linkage groups (LG) using the module *SeparateChromosomes2*. The logarithm of the odds (LOD) and the minimum size for a LG to be formed (*i.e.,* sizeL) were adjusted to LOD=20 and sizeL=10 to maximise the number of markers belonging to a LG. We obtained a total of 40 LGs. Markers were phased in this step (*grandparentPhase=1*) by removing markers without informative (homozygote) grandparents. We used physical positions from the reference genome for marker separation into LGs (*usePhysical = 1*), penalising the markers in different contigs by $\log_{10}(0.01)$. Finally, we used segregation distortion aware LOD scores (*distortionLod=1*). Unassigned markers were subsequently adjoined using the module *JoinSingles2All* with a LOD of 10 and a minimum LOD difference of 5, reaching a total of 3593 markers in the map. The number of newly assigned markers ranged from 6 to 90 per LG, where larger LGs were assigned a higher number of single markers (*e.g.,* LGs 1-3, 5-9 incorporated more than 50 single markers). The *OrderMarkers2* module was used to order and reevaluate the order of the markers within each LG with using the Kosambi function and different recombination rates for males (*recombination1=0.0007*) and females (*recombination2=0.0013*), according to the Arctic charr recombination rates (Nugent et al., 2019; Woram et al., 2003). We again used the physical positions with the same options as in *SeparateChromosomes2*. This module was run five times on each LG to evaluate the order from the immediate previous run. The code of used for this LepMap3 pipeline is freely accessible in: https://github.com/zjons/LepMapWrapper. The linkage maps were visualised using R/LinkageMapView (Ouellette et al., 2018).

QTL mapping

Due to a lower recombination rate in males (Gharbi et al., 2006; Küttner et al., 2011; Ouellet-Fagg, 2023; Woram et al., 2003), the male map was oversaturated (i.e., it contained an increased number of redundant markers). The larger number of recombination events in females allows for a more precise detection of QTL locations thus the female linkage map was used for QTL mapping. The map imported as a *cross* object in r/qtl (Broman, 2009) for QTL mapping. The function *jittermap* was used to adjust the position of overlapping markers and we calculate genotype probabilities with the function *calc.genoprob* was used to (step = 0.1, error.prob = 0.0001) in each family. QTL mapping was performed for each family and each trait using the Haley-Knott method (Knott & Haley, 1992) in the function *scanone.* This function was run with 1000 permutations with and without using *sex* as an additive covariate. We set the 95th, 90th and 80th percentile LOD as significance thresholds to assess different levels of significance of the putative QTL.

To detect QTL with additive or epistatic effect we performed two-loci QTL mapping. For that we first ran the *calc.genoprob* function in each family, this time setting step=0 to skip imputation of pseudomarkers and reduce computational time. The *scantwo* function was then conducted for each family and each trait with n.perms = 1000, to explore the distributions of the five LOD scores $M_f(j,k)$, $M_{fv1}(j,k)$, $M_i(j,k)$, $Ma(j,k)$ and $M_{av1}(j,k)$ (Arends et al., 2010; Broman & Sen, 2009). We then calculated genome-wide significance thresholds for $\alpha = 0.05$ and $\alpha = 0.1$ for those five parameters.

QTL models

Before making the QTL models for each trait and family, we first ran the *calcgeno.prob* (with step = 0.1 and probability of error = 0.0001) function. We used the function *makeqtl* with the genotype probabilities to later test all significant QTL detected for each trait and each family. The function *fitqtl* was subsequently used to study both the percentage of phenotypic variance attributed to each QTL and to calculate the QTL estimated effects (i.e., the difference between the phenotype averages for the heterozygotes and homozygotes (Broman & Sen, 2009)). The resulting QTL models were used to refine the position of each QTL in their respective LG and to recalculate the Bayes credible intervals.

Results

Dense female and male Arctic charr linkage maps were generated

The female and male linkage maps had 3593 markers distributed in 40 linkage groups (LGs). The total length of the female linkage map (3160.388 cM) was substantially larger than of the male map (2007.966 cM). The marker distribution across LGs was homogeneous in the female map, except for a few large areas of low marker density on LG13, LG23 and LG34. The male map on the other hand was characterised markers stacked in close positions, generating both regions with high and low marker density. Hence, the mean distance between markers was larger in the female map ($\bar{x}_{♀}$=0.88 cM) with the largest mean among the LG32 markers (*i.e.,* least marker-dense LG, $\max(\bar{x})_{♀}$ = 3.38 in LG34) and the smallest in LG31 ($\min(\bar{x})_{♀}$= 0.43) compared to the male map ($\bar{x}_{♂}$=0.56 cM where $\max(\bar{x})_{♂}$=1.92 in LG38 and $\min(\bar{x})_{♂}$=0.073 in LG37).

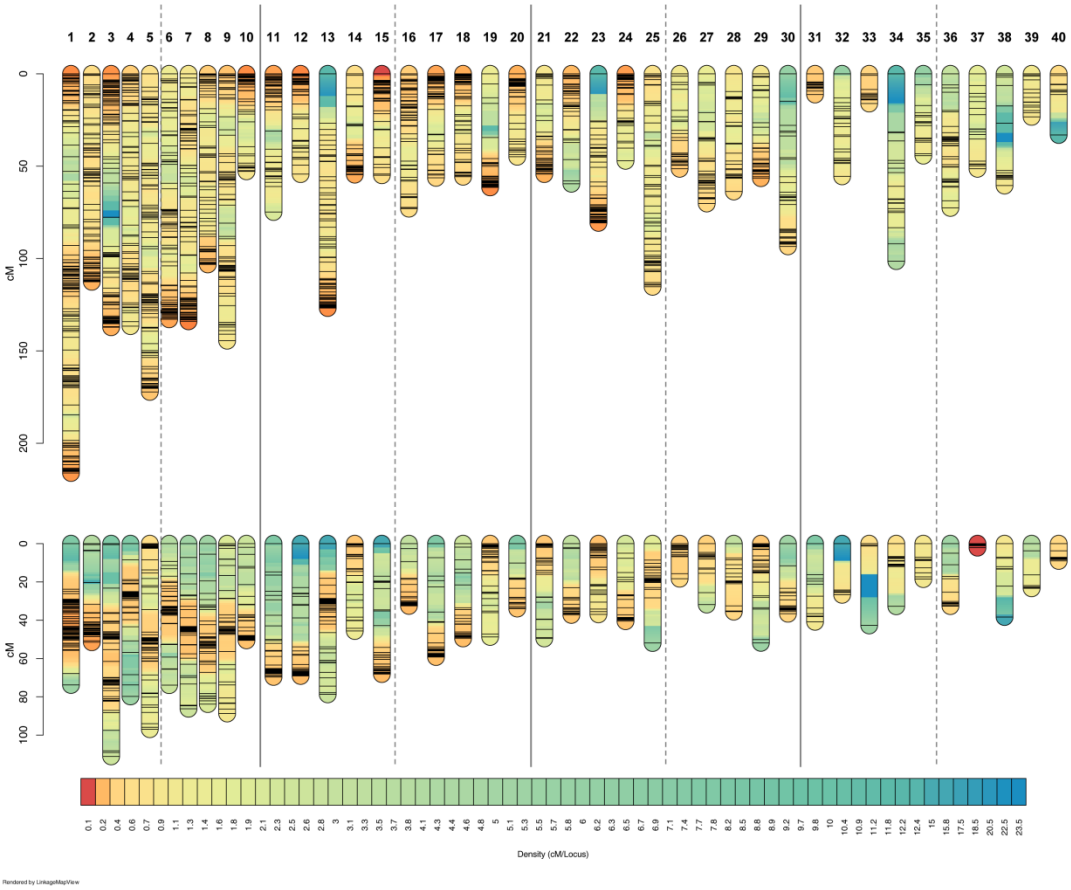

Figure 1. Female (above) and male (below) linkage maps. Warm colours represent areas of high marker density and cold colours represent areas of low marker density. Dark blue represents areas with a marker separation of 30cM or above.

The chromosome repartition of the markers into LGs generally mirrored the chromosomes from the reference genome (**SM – Table 3, SM – Fig. 2**). However, 4 out of the 40 resulting linkage groups (LGs) from the linkage map (LG01, LG03, LG05, and LG06) contained more than one large chromosomal region corresponding to the reference genome (referred to as chromosomes). Most of the remaining LGs contained small chromosome fractions or single markers that either belonged to different chromosomes or were unlocalized markers present in the reference genome. Conversely, 3 chromosomes from the reference genome were split into two or more LGs: chromosome 22 was split into LGs 28 and 31, chromosome 36 into LGs 33 and 34, and chromosome 34 into LGs 35, 38, and 39. To match the linkage map with the chromosomes from the reference genome, we first attempted to split the combined chromosomes into different LGs by progressively increasing the LOD score to 40. While only one LG was split into two appropriate chromosomes, the rest remained combined, and other LGs that previously mapped to their own chromosome were split into two or more blocks.

Tight correlation among linear measurements and PC scores

Most linear traits followed a normal distribution in all families, except for a few traits in the smallest families (H19_101 and H19_106). For detailed density and Q-Q plots for each linear measurement and family, see **SM – Fig. 3-16**.

Geometric morphometric analyses were initially performed on the entire progeny. Mixed-model MANOVA of the Procrustes coordinates revealed that *family*, *log(Csize)*, and their interaction had significant effects on shape variation (**SM – Table 4**). Post-hoc pairwise tests indicated significant differences in mean shape among all families (**SM – Table 5**). However, significant differences in morphological disparity were only observed in the comparisons between H19-101 and H18-05, H19-103, H19-106, and H19-111 (**SM – Table 6**). Despite these significant differences in mean shape, Principal Component Analysis showed considerable overlap among all families, with individuals distributed homogeneously across the morphospace (**SM Fig. 17**). The exception was family H18-05, which formed a distinct cluster across PC1 and PC2 (cumulative explained variance of PC1 and PC2 ≈ 40%). This clustering might be due to different phenotyping times between H18_05 and the rest of the families. Consequently, family H18-05 and the five H19 families were analysed independently.

We used the first 10 PC scores from the PCA on the five H19 families as phenotypic traits for further QTL mapping, as these PC scores explained over 80% of the total phenotypic variation (**SM – Table 7**). Despite being reared in common garden, the fish exhibited differences in body size (**SM – Table 8**), prompting a study of the allometric component of shape. Significant differences were found between the common allometry model (i.e., *coordinates ~ family + size*) and the unique allometry model (i.e., *coordinates ~ family*size*) (p-value = 0.04). This indicates that the interaction between family and size significantly influences shape variation, rejecting the hypothesis of homogeneity of slopes (**SM – Table 9**). We graphically observed these allometric patterns by looking the allometric slopes per family (**SM – Fig. 18**). Given the small effect sizes (Z), the barely significant p-value and the nearly identical allometric slopes in the unique allometries plot, we corrected for common allometric effects in all five H19 families. Principal Component analysis was again performed using the residuals of a simple allometry model (i.e., coordinates ~ size), and the first 10 PC scores were retained for QTL mapping, explaining more than 75% of the cumulative variance (**SM – Table 10**).

The family H1805 was analysed separately using the previously described pipeline, including the correction for allometric effects, as the effect of size on shape was significant ($Z_{(1,49)} = 4.603$, $p = 0.001$, $r^2 = 0.11$). The first 10 PC scores and the first 10 PC scores from the residuals of the simple allometry model were included in the phenotypic matrix for further QTL mapping.

We examined the correlation among all 36 phenotypic traits per family and found consistent correlation patterns across families (**Fig. 2b**). Generally, size was negatively correlated with all 13 linear measurements, PC1 and PC3. Conversely, the 13 linear measurements, PC1 and PC3 were positively correlated with one another. PC2 showed a positive correlation with size and a negative correlation with all the linear measurements. The 10 PC scores obtained directly form the Procrustes coordinates were not correlated with one another, but substantial correlation was observed between these, and the PC scores derived from the residuals between shape and size.

Finally, we investigated the effect of sex on all traits. The six F2 and backcross families contained a total 211 males, 219 females, and the sex of 25 individuals could not be determined (SM - Table 2). Most families adjusted to an approximate 1m:1f ratio, except for the smallest family, H19-106 (N = 17) which had a ratio of 2m:1f. In four out of six families, sex significantly affected size and most linear measurements (**Fig. 2c**). Other PC scores or residuals were significantly affected by sex differently in each family, except for V4, which had a significant effect of sex in three families (**Fig. 2c**).

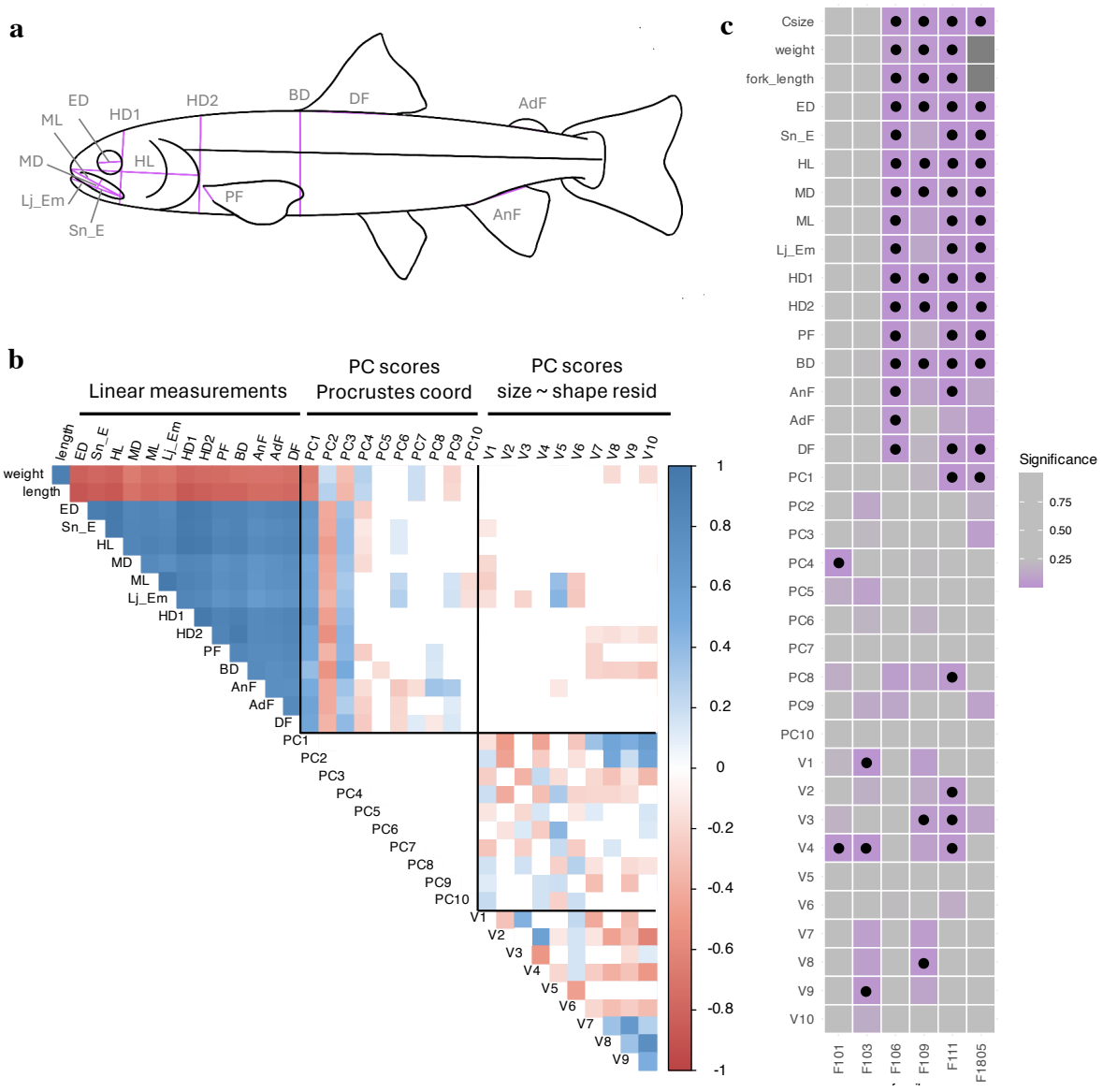

Figure 2. (**a**) Linear measurements used for the phenotypic matrix. ED = eye diameter, Sn_E = snout to eye, HL = head length, MD = maxilla depth, ML = maxilla length, Lj_Em = distance from the lower jaw to end of the maxilla, HD1 = head depth1, HD2 = head_depth2, BD = body depth, PF = depth of the pectoral, AnF = depth of the anal fin, AdF = depth of the adipose fin and DF = depth of the dorsal fin. (**b**) Correlation among all traits from all five H19 families included in the phenotypic matrix (i.e., size traits, linear measurements, PC scores from Procrustes coordinates and PC scores from the residuals of the simple allometry model). Coloured squares represent significant correlation between two traits, using the Pearson correlation. Blue tones depict significant, positive correlations, while red tones depict significant, negative correlations. (**c**) Effects of sex on each trait, per family. Purple colours indicate a p-value < 0.2, and black dots indicate significance (p-value < 0.05). Dark grey squares represent NA data.

QTL were detected for 28 traits in 26 LGs

A total of 57 QTL were found when combining the 36 phenotypic traits traits and the six families. We found significant QTL for 28 out of 36 traits across families (**Fig. 3, SM Table 11**). Overall, significant QTL were found for multiple traits in more than one family, but in shared locations. For instance, QTL explaining variation in maxilla depth and V2 were significant in four families, but the QTL locations were rarely shared across those families (**SM Table 11**). We observed that the number of significant QTL varied greatly across families, this not being linked with cross type or progeny size. For example, only 2 QTL were detected in the family H19_101 (**Fig. 3d, 3f**), while 21 QTL were detected in family H1805, although a great proportion of those 21 traits were strongly correlated with each other (**Fig. 3a**). More than half of the LGs (26 out of 40) harboured at least one QTL in at least one family. Some LGs harboured more QTL than others, for instance LG06, LG08, LG11, LG13 and LG30 harboured at least 4 QTL in different locations (**SM Table 11**).

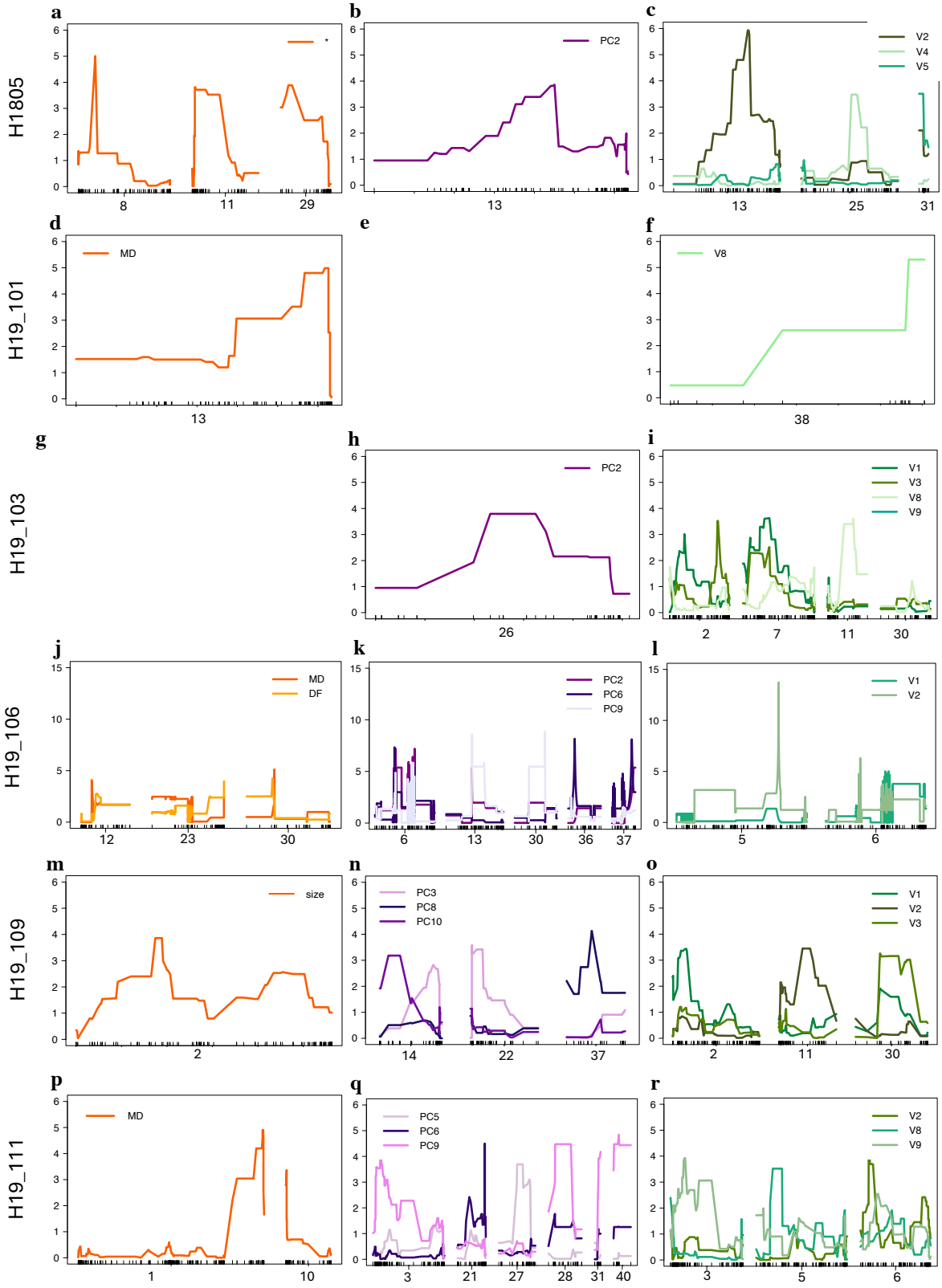

Figure 3. LOD scores of only significant QTL (threshold < 0.2) for all families. The phenotypic traits are divided in their three categories: size traits and linear measurements (orange), PC scores extracted from Procrustes coordinates (in purple tones) and PC scores extracted from the residuals of the Procrustes coordinates against centroid size (green tones). The PC and the V scores in family H1805 were calculated separately and may not

represent the same dimensions of shape variation. The asterisk in plot **a** indicates that the same QTL was detected for multiple linear measurements, and these linear measurements were highly correlated with each other (see **SM – Table 11** and **Fig. 3B**).

Despite all traits extracted from linear measurements being strongly correlated within families, only family H1805 had significant QTL for all of them in the same location. Families H19_101, H19_106 and H19_111 each had at least one significant QTL for maxilla depth, was one of the most variable traits in all families (**SM - Fig. 12**), while no QTL were detected for any of the linear measurements in family H19_103. Many QTL were detected when analysing the PC scores derived from the Procrustes coordinates, especially for families H119_109 and H19_111. In contrast, families H1805 and H19_103 only had a significant QTL for PC2 (**Fig. 3b, h, k, n, q**). PC2 was in fact the most common PC trait with a significant signal for QTL in families H1805, H19_103 and H19_106 albeit in different genomic regions. Although the PC scores for 1805 and the H19 families were extracted separately, the predicted shape changes along PC2 were similar in both cases (**SM – Fig. 19, 20**). The main shape differences along PC2 were in body depth and head morphology, where wider fish had smaller heads, rounder and shorter snouts and smaller eyes, compared to the larger heads, narrower snouts and large eyes, characteristic of the slenderer fish (**SM – Fig. 19, 20**).

Similarly, a substantial amount of QTL were detected for the 10 PC scores extracted from the residuals of Procrustes coordinates and centroid size (traits V1-10). Multiple QTL for V2 were identified in four families (**Fig 3c, l, o, r**), with two of these families (H19_106 and H19_111) sharing a QTL location in chromosome 6 (**Fig 3l, r**). Although their locations were not exactly the same (i.e., 84.5 cM in 106 and 16.6 cM in 111) their Bayes credibility intervals overlapped (**Fig. 4**). Moreover, the trait V2 had the largest LOD score in the entire dataset (family H19_106, **Fig. 3l**). Shape variation along V2 was characterised by substantially wider and longer peduncule area (between the anal fin and the start of the tail of the fish) (**SM Fig. 19**).

A few interacting QTL were detected

Two-QTL genome scans on all families and each trait yielded to two significant interacting QTL. The first one was found in family H19_109 for the trait V2, showing an interaction between chr11@40.8 and chr18@0.2. The second one was found in family H19_111 for trait PC9. In this case, chr20@38.0 significantly interacted with chr28@42.01 and chr40@9.86. The same chromosome 20 interacted with chromosome 31 at a different position (i.e. chr20@16.2 and chr31@11.18).

Low to moderate percentages of PVE were attributed to the detected QTL

In total we detected 33 QTL significantly affecting multiple traits in multiple families. We constructed a QTL model for each family and trait. Most traits were explained by a single QTL model, while one trait was explained by multiple QTL of additive effect and only two were explained by both QTL additive and interactive effects (**Table 2**). All QTL yielded to significantly large LOD scores, and the associated traits accounted for low to moderate

percentages of PVE. QTL models for family H19_106, were characterised by overall low LOD scores, low F values and non-significant p-values, but also with high percentages of PVE (sometimes around 70%). This behaviour suggests the existence of spurious signals, likely due to the low number of individuals in this family. Therefore, QTL models for H19_106 were discarded.

Table 2. Summary of the 25 QTL models run for each trait in each family, excluding family H19_106.

	trait	QTL formula (trait ~ Q)	LOD score	% var	p-value (F)
H1805	size *	Q1 + Q2 + Q3 + Q4	8.879	55.858	0.000
	PC2	Q3	1.621	13.869	0.030
	V2	Q4	6.169	43.346	0.000
	V4	Q5	3.369	26.674	0.001
	V5	Q6	3.369	26.674	0.001
H19_101	MD	Q7	0.773	14.341	0.213
	V8	Q8	3.253	47.867	0.001
H19_103	PC2	Q9	3.644	15.447	0.000
	V1	Q10	3.161	13.546	0.001
	V2	Q11	2.519	10.952	0.004
	V8	Q12	3.629	15.391	0.000
	V9	Q13	3.753	15.873	0.000
H19_109	PC3	Q14	2.852	9.679	0.002
	PC8	Q15	4.365	14.430	0.000
	PC10	Q16	3.350	11.271	0.001
	V1	Q17	2.031	6.995	0.010
	V2	Q18*Q19	4.896	16.036	0.006
	V3	Q20	2.595	8.849	0.003
H19_111	PC5	Q21	3.211	14.999	0.001
	PC6	Q22	3.706	17.102	0.000
	PC9	Q23 + Q24*Q25 + Q26*Q27 + Q28*Q29	17.974	59.731	0.000

V2	Q30	5.007	22.382	0.000
V8	Q31	3.543	16.414	0.000
V9	Q32	3.861	17.749	0.000
MD	Q33	5.757	25.275	0.000

The specific QTL locations and Bayes credibility intervals greatly varied across traits and families (**Fig. 4**). The QTL location of a few traits from different families overlapped. For example, Q10 and Q25 in LG06, Q8, Q13 and Q18 in LG30 and Q6 and Q30 in LG31. In other cases, the QTL locations were close and thus the Bayes credibility intervals greatly overlapped, such as Q2, Q11 and Q15 in LG11, although these regions accounted for different traits in different families. A great proportion of QTL had substantially large Bayes credibility intervals, often spanning the whole LG, such as Q26 in LG10, Q27 in LG21 and Q19 in LG37 (**Fig. 4**).

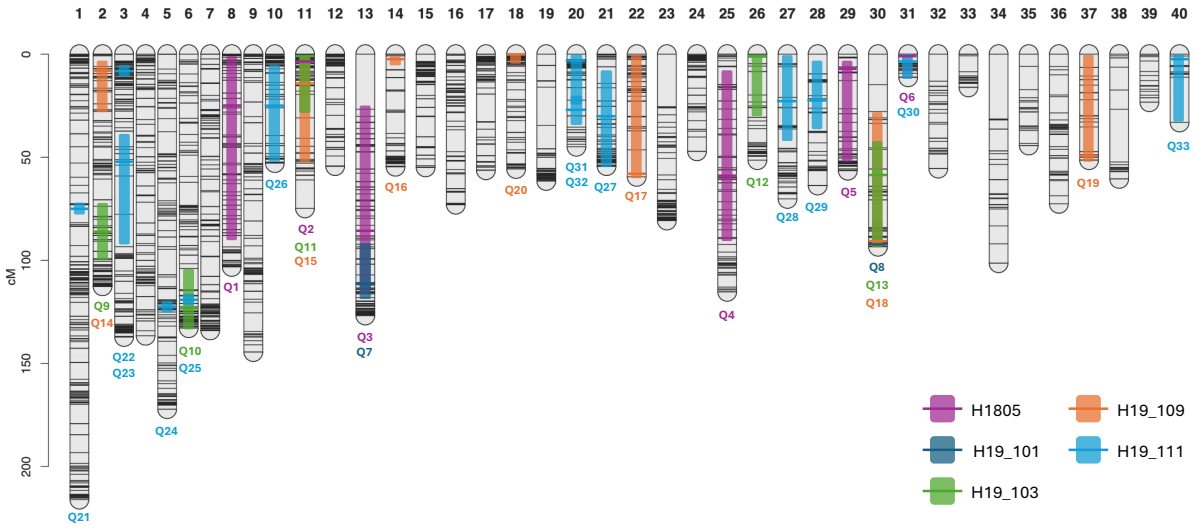

Figure 4. Female linkage map containing significant QTL for each family. Black horizontal lines represent the markers. Thick, coloured horizontal lines show the refined QTL location following the QTL models. Coloured vertical boxes indicate the span of the Bayes credibility intervals for each QTL. Each colour represents one family, and the QTL identification numbers below the LGs (i.e., Q1 – Q33) are the same as in **Table 2.**

Discussion

We generated a high-resolution linkage map from two natural sympatric Arctic charr populations, opening the door to the exploration of the genomic architecture underlying phenotypic traits in a context of ecological diversification. Our QTL mapping analyses revealed that numerous morphological traits associated with benthic or limnetic ecologies have a genetic basis. Notably, these morphological traits were predominantly explained by a single QTL with moderate to high percentages of PVE. QTL for these traits were sparsely distributed across the genome, with few instances of colocalization. The most consistent QTL detected across families and/or genomic region explained shape differences related to the relative size of the head, maxilla shape, and peduncle depth.

A dense, high-quality Arctic charr linkage map

We present a high-resolution Arctic charr linkage map constructed from parental lines belonging to two sympatric populations and mapped morphological traits related to benthic and limnetic habitat specialisations. We highlight the value of using parental lines from the wild and a reasonable number of families, given the long generation times in Arctic charr. Among previous QTL studies with focus on these and other "ecological traits" (e.g., (Laporte et al., 2015; Rogers & Bernatchez, 2005)), few have utilised wild parental lines and tend to restrict their sample size and genetic diversity to one or two families (reviewed by (Ashton et al., 2017)). The prior QTL mapping effort on Icelandic Arctic charr used wild parental lines form two different locations, which were crossed to the same morph to the first generation (Ouellet-Fagg, 2023). Even though two Arctic charr morphs from one population were used in our study, we increased both the number and the diversity of recombination events by first generating hybrids (F1), which were later crossed to the second generation. This crossing design yielded four different cross types, facilitating the exploration of various QTL effects for the same traits.

Our linkage map included around 3600 markers distributed in 40 LGs, corresponding to the same number of chromosomes in the reference genome. The differences between the male and the female consensus maps were consistent with previous salmonid maps (Gharbi et al., 2006; Küttner et al., 2011; Ouellet-Fagg, 2023). As mentioned above, the reference genome was based on the large benthic (LB) morph from lake Thingvallavatn, which has evolved in sympatry in the last 10,000 years and currently represents a single genetic population (Guðbrandsson et al., 2019), de la Cámara et al., *in prep*). The LGs in our map did not perfectly match the chromosomes from the reference genome, likely due to the fact that the assembly of the genome is not yet completed. The closest available reference genome is based on *Salvelinus malma* (Christensen et al., 2018) from the Arctic rather than the Atlantic lineage. Due to large chromosomal rearrangements (CR) between major lineages (Hale et al., 2021; Pomianowski & Ocalewicz, 2021), the *S. malma* genome was not used for this study.

Highly conserved trait correlation

Correlation tests conducted on the phenotypic matrix revealed strong positive correlations among all linear measurements. Note that the 13 linear measurements were targeted to capture traits typically associated with benthic and limnetic adaptations present in the parental lines (e.g., in the wild benthic fish have deeper heads and bodies, rounded snouts, deeper maxillae, smaller eyes and deeper pectoral fins, while limnetic fish have more elongated heads and bodies, pointier snouts, slender maxillae, larger eyes and narrower pectoral fins). While one may expect a breakdown of trait correlation on the F2 and BC1 progenies, our findings show that trait correlation is strongly conserved (i.e., integration, or limited modularity). This integration of traits into a single covariance structure is however not observed in intra- or inter-morph crosses (F1) between the same Arctic charr morphs, reared in common garden conditions and phenotyped across development (11 weeks after the onset of exogeneous feeding was the latest phenotyping point) (Horta-Lacueva et al., 2021). The reason may be that trait correlation increases across development, given that the F2 and BC1 progenies were phenotyped much later (i.e., 14 and 18 months after fertilisation). Furthermore, the linear measurements were highly correlated with body size measurements such as centroid size, length and weight, even after accounting for size. This indicates that allometric effects are crucial for understanding shape variation across development, even within individuals of the same age (de la Cámara et al., 2023; Horta-Lacueva et al., 2021; Ouellet-Fagg, 2023).

Hierarchical genomic architecture and limited pleiotropic effects

We found that most traits were explained by a single QTL with moderate to high percentages of PVE. We found limited examples of QTL colocalization across traits and families, suggesting a variable genetic architecture behind the phenotypic traits included in this study. There are three, non-mutually exclusive reasons why we should not directly assume a monogenetic architecture. The most likely one is that undetected loci of small effects may modulate the larger effects of the detected QTL, suggesting a hierarchical genomic architecture. The second reason is a technical one: to confidently ensure that a QTL exist, we implemented a genome-wide scan, consisting of setting fixed significance thresholds for QTL detection across the genome. If a chromosome-by-chromosome scan had been performed, it is possible that the same trait would have been explained by multiple QTL of small additive effects, in concordance with Ouellet's (2023) results. The third reason is the inherent complexity of measuring shape variation. In this study the phenotypic matrix was partly redundant, where not targeted measurements (i.e., PC scores) captured traits explained by targeted measurements (i.e., linear measurements), and the residual PC scores (Vs) captured both linear measurements and PC scores in different ways. The different ways of capturing body depth, often explored as a trait traditionally associated to benthic-limnetic adaptations, are an illustrative example of the complexity of studying the genetic component of shape variation: 12 traits within the phenotypic matrix explained different aspects of shape variation in body depth (e.g., BD (body depth), PC1 to PC5 and V1 to V6, with body depth varying in different parts of the body and in different directions). Nine out of those 12 traits were explained by at least one QTL in 12 different LGs, suggesting that the combination of different QTL explaining similar phenotypic traits could contribute to a presumable polygenic basis.

QTL were present in 23 out of 40 LGs (when excluding those QTL detected in family H19_106). Seven of these 23 LGs harboured two or more QTL, and in five of those seven,

their credibility intervals overlapped, suggesting pleiotropic effects. For example, LG31 harboured QTL for V2 and V5 (traits related to body depth, snout length and peduncle depth), explaining similar percentages of phenotypic variance. In other cases where overlapping QTL accounted for two traits, one had a large impact on overall shape variation while the second one only accounted for small percentages of shape variation. This is the case of Q10 and Q25, which had a similar position on LG06, and were responsible for traits V1 and PC9. While shape variation in V1 involved large shape variation in snout orientation, maxilla orientation, pectoral fin orientation and body depth, changes associated to PC9 were subtle, and mostly associated to caudal length and gill orientation. A similar case concerns LG30.

Implications in the Arctic charr ecological diversification

The most consistent QTL detected across families and/or locations explained shape differences related to the relative size of the head, maxilla shape, and peduncle depth. For instance, QTL were detected for two traits in multiple families from different cross types. One of them was PC2 in families H1805, H19_103 and H19_106. Shape differences along PC2 explained one of the most important characteristics of benthic and limnetic adaptations: the relative size of the head. The SB charr from lake Thingvallavatn have larger heads relative to their bodies (de la Cámara et al., 2023; Skúlason et al., 1989), which have likely arisen through paedomorphism, an evolutionary mechanism where a population or species tends to retain juvenile traits (Eiríksson et al., 1999; Skúlason et al., 1989). Our data seems to confirm this: PC2 is positively correlated with length and weight, and the predicted shapes for lower values of PC2 have larger heads and rounder snouts, resembling the small benthic morphology. On the other hand, predicted shapes for high values of PC2 correlate with pointier snouts and smaller heads in relation to the body, resembling the planktivorous-like charr morphology.

Another trait whose variance is attributed to multiple QTL in multiple families is V2. Shape variation along the V2 eigenvector was mostly explained by morphological changes in the peduncle area. Previous studies have shown that variation in the shape of the peduncle has a strong genetic component in allopatric populations of Arctic charr (Janhunen et al., 2009). Ecologically, shape variation in the peduncle is mostly attributed to swimming performance (Blake, 2004). While a study in lake charr discusses that short and thick peduncles are associated with buoyancy regulation and thus help improve swimming abilities along the open water column (Muir et al., 2016), other studies on Arctic charr agree that elongated body shapes with slim caudal peduncles help to reduce drag and improve acceleration in open waters (Skoglund et al., 2015). In Icelandic Arctic charr, thick peduncles have been associated with deeper bodies and relatively small heads, generally characteristic of adaptations to benthic environments (Kristjánsson et al., 2012).

Only one trait, V8 had significant QTL in the three families belonging to the same cross type (i.e., a backcross to a SB grandparent in H19_101, H19_103 and H19_111), however the QTL locations differed (in LG38, LG11 and LG5, respectively). This may be due to the different relative frequencies of weaker and stronger QTL. Shape variation in V8 affected mostly maxilla length and to some extent maxilla depth. Although V8 accounted for subtle changes in shape variation (3.75% of the residuals of shape against size), its QTL contributed to a large proportion of the V8 phenotypic variance (e.g., 47.86% PVE in H19_101). Furthermore, other QTL related to maxilla shape (maxilla depth, MD) were also detected in multiple families. Due to the fact that all the linear traits were strongly

correlated with one another, only one of the most variable traits, maxilla depth (MD), was explained by QTL in three families in different genomic regions. A detailed study on shape variation of bone structures in the four Arctic charr morphs from Thingvallavatn also reported significant differences in maxilla depth between SB and PL, with PL having a thinner maxilla structure both in the middle of the bone and at the caudal lobe (Jónsdóttir et al., 2024). In this publication, Jónsdóttir and colleagues discuss how, even though the maxilla is a movable element within the salmonid's craniofacial structure, its role in feeding is still unexplored.

Briefly, most of the phenotypic traits were explained by a single QTL and the percentages of PVE ranged from modest to high, pointing towards a hierarchical genomic architecture. Integrating the results from multiple cross types and families allows for a more complete picture, where we see that multiple QTL account for different dimensions of shape, likely playing different roles in the same network, or in different networks with similar effects on shape (i.e., canalisation, discussed in (Horta-Lacueva et al., 2023)). Another important consideration involves that multiple genes may be covered by those detected QTL with large credibility intervals. Because rapid trait divergence in early stages will facilitate reproductive isolation in sympatric populations, the larger QTL effects may have enhanced the rapid divergence of the SB and PL charr, later modulated by genome-wide QTL of smaller effects and likely phenotypic plasticity (Parsons et al., 2011). Additionally, the fact that following the colonisation of the lake, populations of Arctic charr underwent a micro-allopatry period may have contributed to the reproductive isolation between SB and PL and thus helped strengthening the differences between the two morphs (Brachmann et al., 2022; Kapralova et al., 2011).

QTL mapping studies the effects of a mutation on a phenotype, but it does not help us determine which alleles at specific loci will be employed in adaptive evolution (Barghi et al., 2020). Thus the shape variation attributed to the benthic and limnetic parental lines may not reflect adaptations to these niches. While QTL mapping has been applied in other freshwater systems to study ecological traits (Laporte et al., 2015; Ouellet-Fagg, 2023), causative genes in adaptive divergence are rarely identified. One way to address this challenge is by incorporating quantitative expression data (Gagnaire et al., 2013), and/or combining the results from QTL mapping with selection signatures from wild populations (Albertson et al., 2014; Laporte et al., 2015; Rogers & Bernatchez, 2005). Furthermore, one may predict that detecting causative genes for adaptive divergence would involve a genomic region accounting for variation in multiple traits (i.e., a case of extreme pleiotropy), where a "magic QTL" under selection is able to switch on and off "the benthic form" or "the limnetic form". This would theoretically strengthen pre-zygotic reproductive barriers. Although examples of highly pleiotropic genes exist in unicellular organisms (Kurlandzka et al., 1991; McGee et al., 2016; Wang et al., 2010), pleiotropy at high levels has been shown to be constraining in multicellular organisms (such as flowering plants, *Drosophila*, *Heliconius* butterflies or sticklebacks) while low or intermediate levels of pleiotropy may be beneficial for adaptation (Frachon et al., 2017; Fraïsse et al., 2019; Greenwood et al., 2016; Lewis et al., 2019; Martin & Orgogozo, 2013; McGee et al., 2016; Mckay et al., 2003; Mills et al., 2014; Rennison & Peichel, 2022). Moreover, detecting signatures of selection in QTL may be further challenged by weak selection pressures and/or loci of smaller effects involved in variable genetic architectures. Future efforts to understand the genetic architecture of ecological adaptations should include replicate populations exposed to similar selection pressures, both in the laboratory and (ideally) in a

natural setting, in combination with carefully designed QTL experiments that include a large number of families and individuals. Despite the feasibility of such experiments in model organisms, the application to non-model organisms presents a long and complex road ahead, especially when sampling and maintenance in captivity is challenging. Our study aims to contribute to overcoming these challenges by providing insights that may help streamline research in these less studied systems.

Funding

This work was fully funded by the Icelandic Centre of Research, RANNÍS (Icelandic Research Fund grant no. 1535–1533090) to KHK, the University of Iceland‘s Doctoral Fund to MC and the Aðalsteinn Kristjánsson Memorial Fund from the University of Iceland to MC.

Ethical statement

Fish collection was obtained with permissions from the farmers in Mjóanes, and from the Thingvellir National Park Commission. Ethics committee approval is not needed for regular or scientific fishing in Iceland (The Icelandic law on Animal protection, Law 15/1994, last updated with Law 55/2013). All fishing was done with permits from the Directory of Fisheries, SSS held active permits for the sampling period.

The rearing and the experimental work were conducted in the facilities of Hólar University Aquaculture Research Station, which has an operational license under the Icelandic Aquaculture law (Law No. 71/2018). This law includes clauses of best practices for animal care and experimental work.

Conflicts of interest

The authors declare no conflict of interests.

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Supplementary tables

SM – Table 1. Primer sequences used for sexing the F2 and BC specimens (SdY) and its control (ETBR2).

Primer name	Sequence
SdY-F2 (forward)	TTG GGC CTA TGA ATT TCT GAT GTT G
SdY-R4 (reverse)	TTC ATA TCA CTC ACC CTG TCT GAA G
SdY-F3 (forward)	TTC AAT GGC TGA CAG AGA GGC CAG A
SdY-R5 (reverse)	GTG AAA TCT GTT GTG AAT TAC CCG T
ETBR2-F (forward)	GAG CTG TCC TTG GCT TTG TC
ETBR2-R (reverse)	ACG CCC TGG TCA TCA ACT AC

SM – Table 2. Distribution of males and females in the progeny of each family (i.e., excluding parents and grandparents).

Family	Females	Males	NA
H18-05	22	29	0
H19-101	18	18	0
H19-103	55	46	3
H19-106	5	10	0
H19-109	67	66	8
H19-111	52	42	14
Total	219	211	25

SM – Table 3. Chromosome repartition of the LG obtained in the present linkage map in relation of the reference genome (chr ref).

LG	chr ref	count
1	1	216
	30	80
	23	2
	27	1
	29	1
	unloc	5
2	3	164
	36	1
3	13	104
	26	81
4	5	124
	35	5
	unloc	1

5	10	116
	40	40
	3	2
	8	1
	22	1
	unloc	5
6	11	103
	25	26
	38	5
	24	1
	unloc	5
7	2	148
	37	6
	7	1
	unloc	9
8	6	131
	21	1
	unloc	3
9	4	126
	32	10
	unloc	3
10	15	89
	4	1
	unloc	2
11	18	85
	unloc	3
12	29	99
13	7	139
	2	1
	33	1
	unloc	3
14	14	88
15	24	102
	26	1
16	8	99
	unloc	4
17	12	96
18	23	93
	15	1
	39	1
	unloc	3

19	21	83
	27	1
20	16	78
21	28	74
	unloc	5
22	20	72
	unloc	1
23	27	100
	8	1
	18	1
24	17	81
	20	1
	unloc	2
25	9	78
	39	2
	16	1
	32	1
	unloc	5
26	19	59
	10	1
27	35	49
	5	6
28	22	52
	17	1
	unloc	1
29	31	76
30	38	35
	25	10
	unloc	5
31	22	26
32	39	26
	9	1
	unloc	2
33	36	27
	3	2
	unloc	2
34	36	26
	39	3
	unloc	1
35	34	20
36	37	35

	2	4
	7	1
	unloc	3
37	32	26
	4	2
	unloc	1
38	34	19
	unloc	1
39	34	16
	30	1
	unloc	1
40	22	23

SM – Table 4. Significant terms affecting shape individuals in all six families in a mixed-model MANOVA using residual randomization (i.e., *coords ~ family * log(Csize)*). Sums of Squares and Cross-products: Type III. Significance codes: 0 = (***); < 0.001 = (**); < 0.01 (*); < 0.05 (.).

Term	**Df**	**SS**	**MS**	**Rsq**	**F**	**Z**	**Pr (>F)**
family	4	0.00413	0.00103	0.016	2.019	3.077	0.002 (**)
log(Csize)	1	0.00643	0.00643	0.024	12.567	5.475	0.001 (**)
family:log(Csize)	4	0.00401	0.00100	0.015	1.961	2.972	0.002 (**)
Residuals	387	0.19801	0.00051	0.757			
Total	396	0.26173					

SM – Table 5. Pairwise distances between mean shapes for all six families when fitting a reduced model for size (i.e., *coords ~ log(Csize)*). N(perm) = 10,000. . Significance codes: 0 = (***); < 0.001 = (**); < 0.01 (*); < 0.05 (.).

Family pair	Distance between means	UCL (95%)	Z	p-value
H1805 : H19-101	0.0303	0.0102	5.567	0.001 (**)
H1805 : H19-103	0.0314	0.0074	7.583	0.001 (**)
H1805 : H19-106	0.0313	0.0113	5.052	0.001 (**)
H1805 : H19-109	0.0256	0.0066	7.027	0.001 (**)
H1805 : H19-111	0.0225	0.0073	5.597	0.001 (**)
H19-101 : H19-103	0.0167	0.0073	4.677	0.001 (**)
H19-101 : H19-106	0.0184	0.0108	3.433	0.001 (**)
H19-101 : H19-109	0.0107	0.0076	3.105	0.002 (**)
H19-101 : H19-111	0.0137	0.0079	3.651	0.001 (**)
H19-103 : H19-106	0.0210	0.0094	4.666	0.001 (**)
H19-103 : H19-109	0.0134	0.0045	6.064	0.001 (**)
H19-103 : H19-111	0.0131	0.0053	5.285	0.001 (**)
H19-106 : H19-109	0.0167	0.0095	3.821	0.001 (**)
H19-106 : H19-111	0.0186	0.0098	3.999	0.001 (**)
H19-109 : H19-111	0.0076	0.0050	3.549	0.001 (**)

SM – Table 6. Pairwise distances between morphological disparity values (i.e., shape variance) for all six families when fitting a reduced model for size (i.e., *coords ~ log(Csize)*). N(perm) = 10,000. . Significance codes: 0 = (***); < 0.001 = (**); < 0.01 (*); < 0.05 (.).

Family pair	Distance between variances	UCL (95%)	Z	p-value
H1805 : H19-101	2.93e-04	2.49e-04	1.975	0.021 (.)
H1805 : H19-103	5.91e-05	1.88e-04	-0.050	0.531
H1805 : H19-106	2.87e-05	3.51e-04	-1.007	0.836
H1805 : H19-109	1.10e-04	1.83e-04	0.803	0.218
H1805 : H19-111	5.58e-05	2.07e-04	-0.108	0.552
H19-101 : H19-103	2.34e-04	2.05e-04	1.795	0.030 (.)
H19-101 : H19-106	2.64e-04	3.57e-04	1.183	0.115
H19-101 : H19-109	1.83e-04	2.03e-04	1.362	0.080
H19-101 : H19-111	3.49e-04	2.30e-04	2.378	0.005 (*)
H19-103 : H19-106	3.04e-05	3.34e-04	-1.045	0.857
H19-103 : H19-109	5.13e-05	1.42e-04	0.092	0.466
H19-103 : H19-111	1.15e-04	1.71e-04	0.948	0.175
H19-106 : H19-109	8.17e-05	3.23e-04	-0.096	0.557
H19-106 : H19-111	8.45e-05	3.49e-04	-0.142	0.567
H19-109 : H19-111	1.66e-04	1.66e-04	1.628	0.051

SM – Table 7. Percentage of variance explained by the first 19 eigen-values resulting from the principal component analysis of Procrustes coordinates from the progeny of all five H19 families.

Dimension	Variance percentage (%)	Cumulative variance percentage (%)
1	22.68	22.68
2	14.15	36.37
3	11.20	48.51
4	8.28	56.68
5	6.10	62.63
6	5.07	67.59
7	3.66	71.24
8	3.20	74.36
9	2.95	77.33
10	2.55	79.80
11	2.29	82.09
12	1.61	83.77
13	1.55	85.35
14	1.45	86.84
15	1.31	88.12
16	1.13	89.23
17	1.02	90.28

SM – Table 9. Analysis of shape variance between the common allometry model (*i.e., coordinates ~ family + size*) and the unique allometry model (*i.e., coordinates ~ family * size*), using residual randomization. N(perm) = 1000.

Model	ResDf	Df	RSS	SS	MS	Rsq	F	Z	P Pr(>F)
Common allometry (null)	387	1	0.186			0.000			
Unique allometry	383	4	0.182	0.00327	0.00081	0.0134	1.718	2.630	0.04 (*)
Total	392		0.245						

SM – Table 10. Percentage of variance explained by the first 19 eigen-values resulting from the principal component analysis of the residuals from the Procrustes coordinates from the progeny of all five H19 families against their size.

Dimension	Variance percentage (%)	Cumulative variance percentage (%)
1	20.37	20.37
2	13.94	34.31
3	10.29	44.60
4	7.27	51.87
5	6.17	58.04
6	5.42	63.46
7	4.14	67.59
8	3.75	71.35
9	3.38	74.72
10	2.89	77.61
11	2.31	79.92
12	1.87	81.79
13	1.75	83.53
14	1.67	85.21
15	1.42	86.62
16	1.19	87.82
17	1.14	88.96
18	1.11	90.06

SM – Table 11. Detected QTL per family for each trait. The rows represent the mapped traits, while the columns represent each family. If a QTL exists, its location and significance is indicated as: chr number : position (cM) and significance level, ranging from 0.01 (***), 0.05 (**) and 0.1 (*).

Trait / family	101	103	106	109	111	1805
Csize				2 : 34.865 **		8 : 18.4 **
Fork length				2 : 34.865 *		
weight				2 : 34.865 **		
ED						8 : 18.1 *
Sn_E						
HL						8 : 17.7 **
MD	13 : 5.259 **		12 : 16.288 *		1 : 214.6 ***	11 : 4.9 *
					10 : 1.1 **	
ML						8 : 16.8 ***
						29 : 58.26 ***
Lwj_endM						8 : 16.8 **
						19 : 58.26 **
HD1						8 : 18.0 **
HD2						8 : 18.3 **
						11 : 5.3 **
PF						8 : 18.88 **
						11 : 5.3 **
BD						8 : 18.3 ***
						11 : 5.3 ***
						29 : 14.9 ***
AF						
AdF						
DF			23 : 45.5 **			6 : 73.5
			30 : 67.3 **			
PC1						
PC2		26 : 32.6 *	6 : 34.3 **			13 : 86.3 ***
PC3				22 : 4.2 *		
PC4						
PC5					27 : 41.1 *	
PC6			36 : 14.5 **		21 : 52.55 ***	
			37 : 43.4 **			
PC7						
PC8				37 : 23.7 ***		
PC9			13 : 57.4 *		03 : 15.0 ***	
			30 : 67.2 *		28 : 43.4 ***	

					31 : 11.176 ***	
PC10				14 : 5.6 *		
V1		7 : 45.3 **	5 : 134.9 *	2 : 15.3 *		
V2			**6 : 84.5 *** **	11 : 41.7 *	**6 : 16.6 *** **	13 : 86.3 ***
V3		2 : 91.0 *		30 : 31.69 *		
V4						25 : 67.2 *
V5						31 : 2.6 *
V6						
V7						
V8	38 : 60.48 **	11 : 48.09 *			5 : 48.9 *	
V9		30 : 75.4 *			3 : 27.571 **	
V10						

Supplementary figures

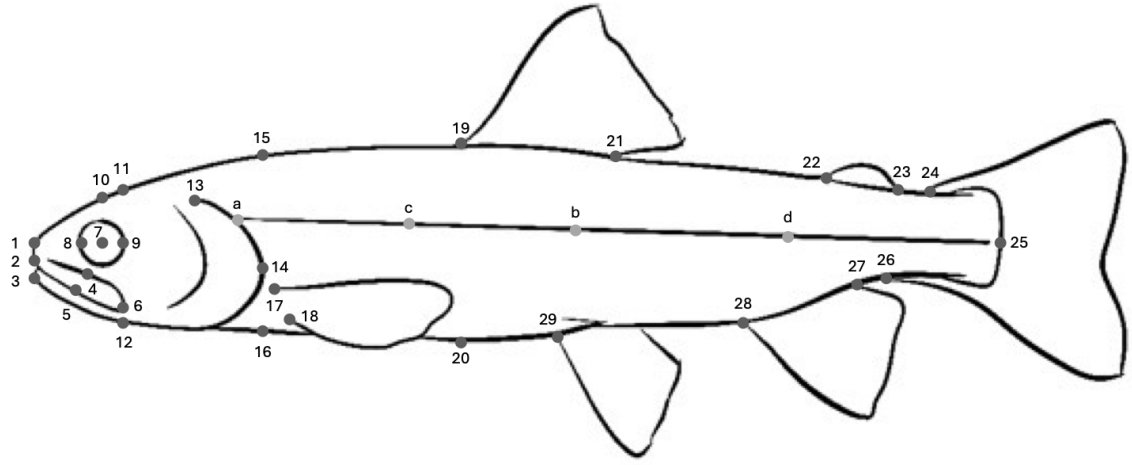
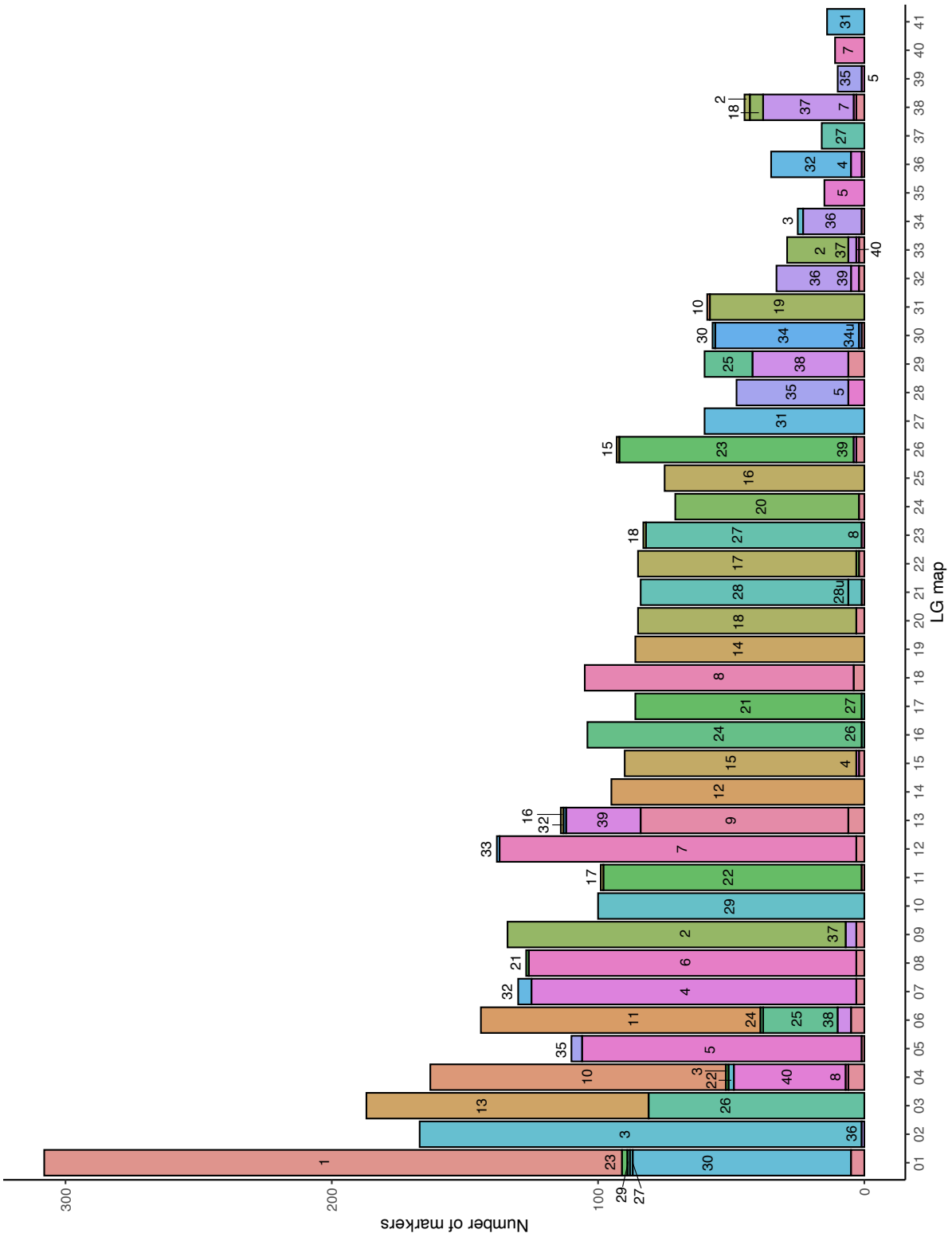
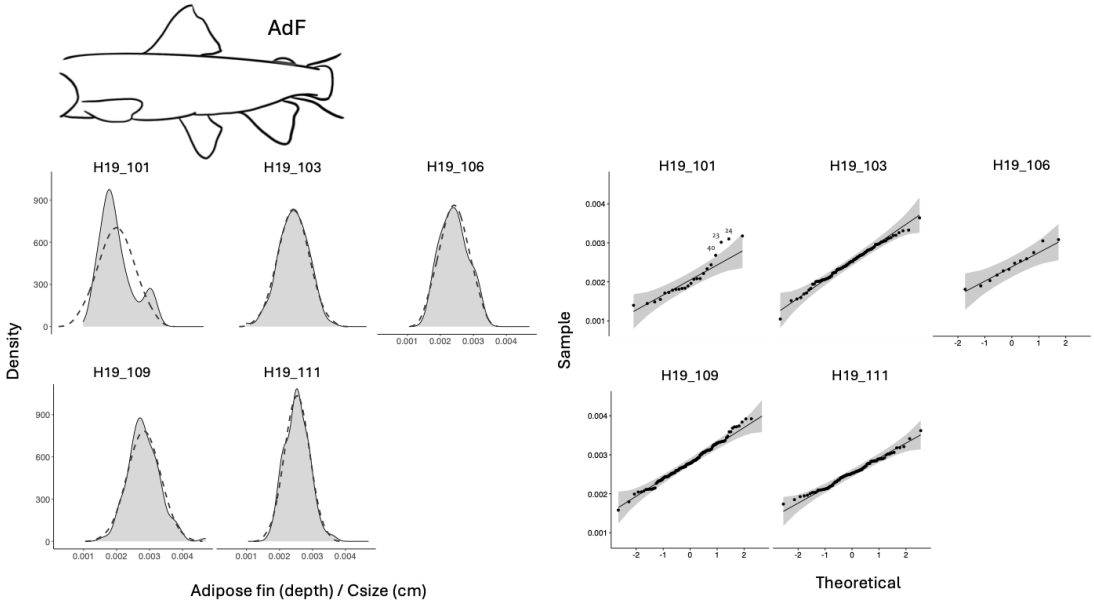


Figure 1. Landmarks taken for geometric morphometrics analyses and linear measurements. Numbers repesent the landmarks used for the analyses (in dark grey), while letters (in light grey) represent landmarks along the lateral line used for unbending. **1** – Tip of the snout, **2** – tip of the upper jaw, **3** – tip of the lower jaw, **4** – most dorsal end of the maxilla, **5** – most ventral end of the maxilla, **6** – posterior tip of the maxilla, **7** – centre of the eye, **8** – most anterior edge of the eye, **9** – most posterior edge of the eye, **10** - orthogonal projection of landmark 7 on the dorsal edge of the fish, **11** - orthogonal projection of landmark 9 on the dorsal edge of the fish, **12** - orthogonal projection of landmark 9 on the ventral edge of the fish, **13** – gill opening, **14** - most posterior end of the gill opening, **15** - orthogonal projection of landmark 14 on the dorsal edge of the fish, **16** - orthogonal projection of landmark 14 on the ventral edge of the fish, **17** - dorsal insertion of the pectoral fin, **18** - ventral insertion of the pectoral fin, **19** - anterior insertion of the dorsal fin, **20** - orthogonal projection of landmark 19 on the ventral edge of the fish, **21** - posterior insertion of the dorsal fin, **22** - anterior insertion of the adipose fin, **23** - posterior insertion of the adipose fin, **24** - dorsal insertion of the caudal fin, **25** - end of the spinal column, **26** - ventral insertion of the caudal fin, **27** - posterior insertion of the anal fin, **28** - anterior insertion of the anal fin, **29** - anterior insertion of the pelvic fin, **a** - meeting point between the gill opening and the lateral line, **b** - middle point between landmark 25 and landmark a on the lateral line, **c** - middle point between a and b on the lateral line, **d** - middle point 25 and c on the lateral line.

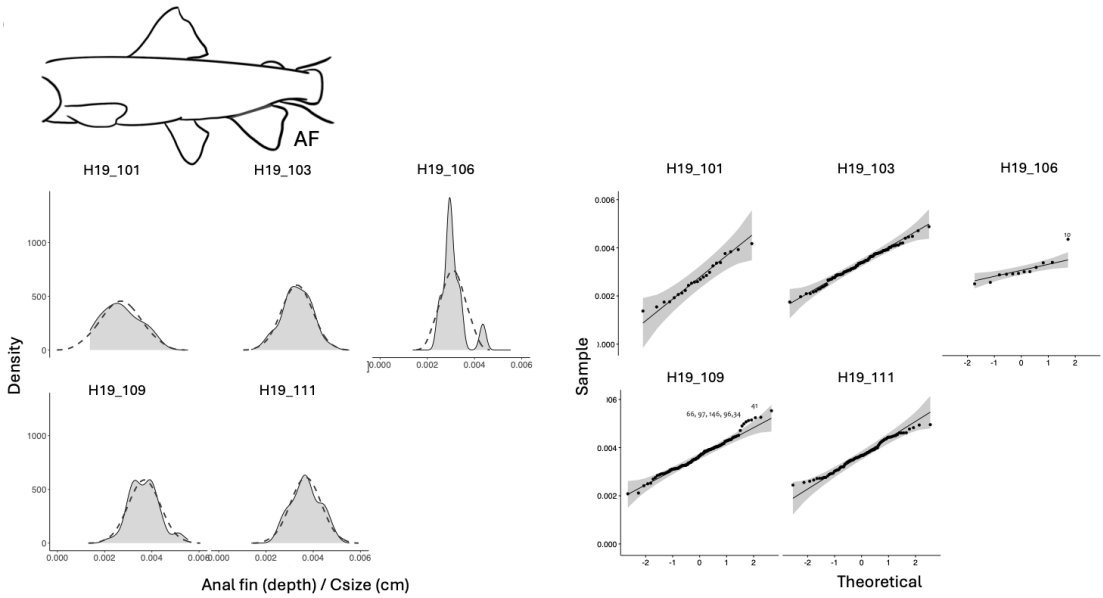
The linear measurements extracted from landmarks: eye diameter (**ED**, 8-9), snout to eye (**Sn_E**, 1-8), head length (**HL**, 1-14), maxilla depth (**MD**, 4-5), maxilla length (**ML**, 2-6), distance from the lower jaw to end of the maxilla (**Lj_Em**, 3-6), head depth1 (**HD1**, 11-12), head_depth2 (**HD2**, 15-16), body depth (**BD**, 19-20), and depth of the pectoral (**PF**, 17-18), anal (**AF**, 27-28), adipose (**AdF**, 22-23) and dorsal fins (**DF**, 19-21).



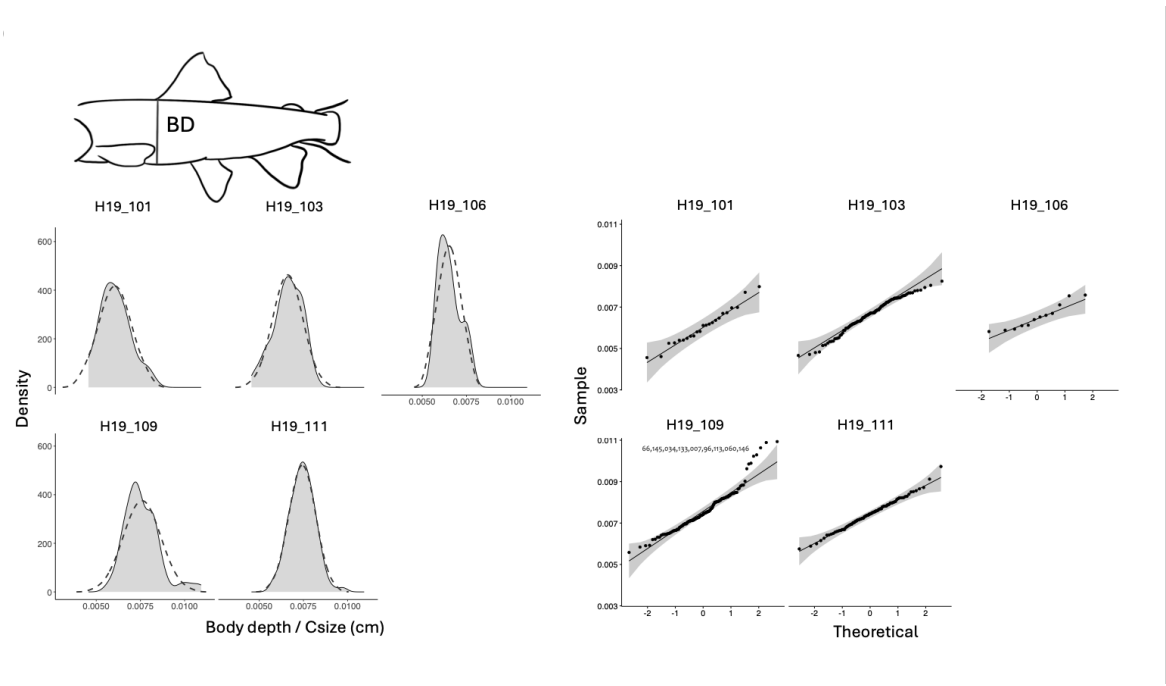
SM – Figure 2. Chromosome repartition of the linkage map with reference genome, whose assemblage was based on the large benthic (LB) morph from Thingvallavatn. Each bar on the X axis represents a linkage group (LG) from the linkage map. The different colours and numbers in the bars represent the chromosome of the reference genome that the markers belong to. Unlabeled red boxes represent unocalised markers from the reference genome.



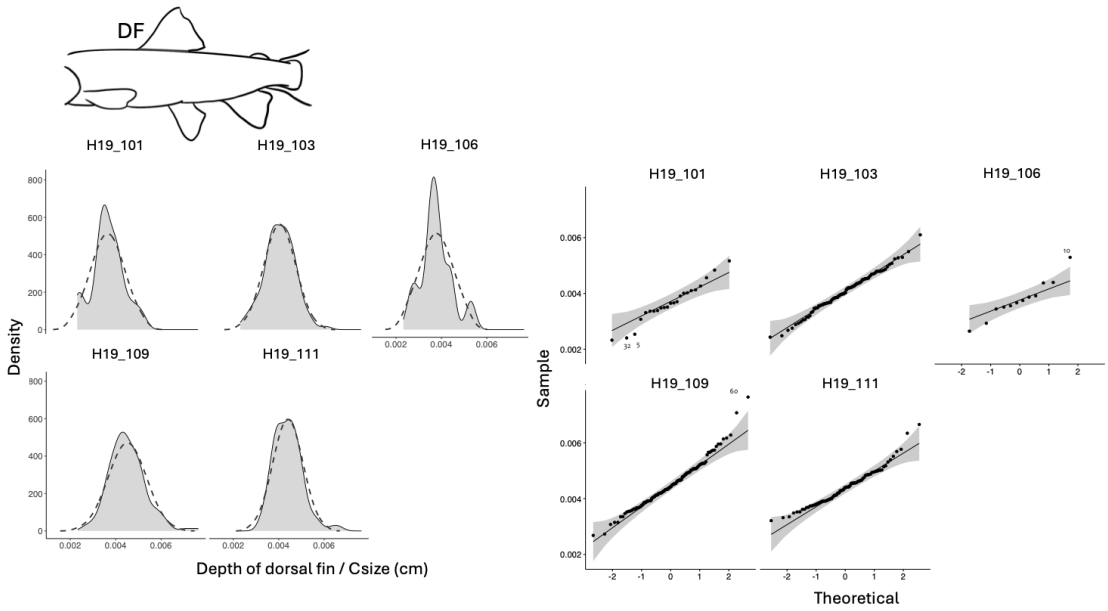
SM – Figure 3. Density distribution and Q-Q plots of *depth of adipose fin* with outliers.



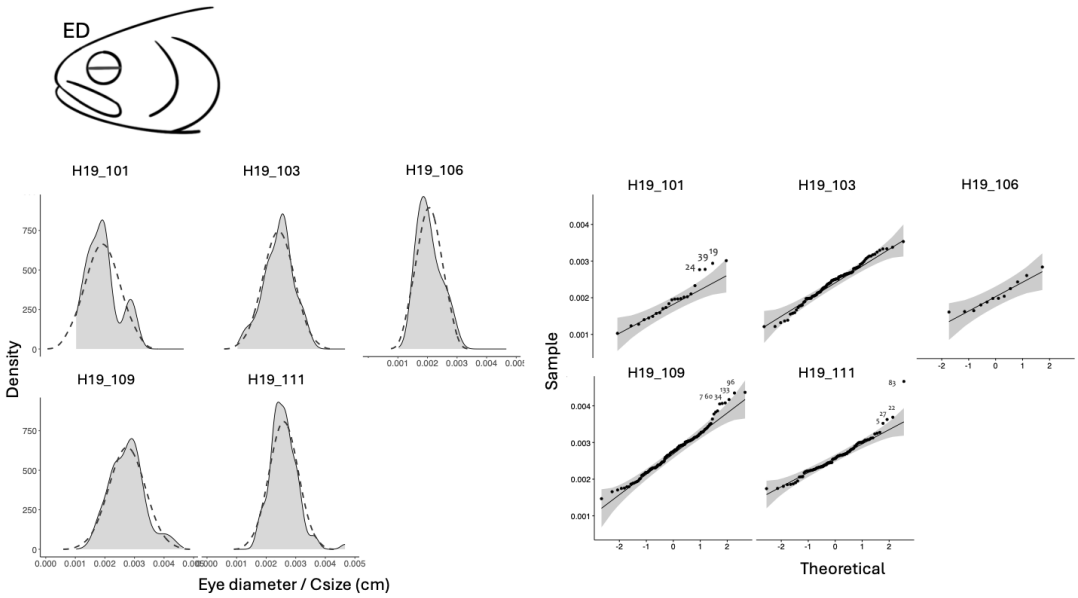
SM – Figure 4. Density distribution and Q-Q plots of *depth of anal fin* with outliers.



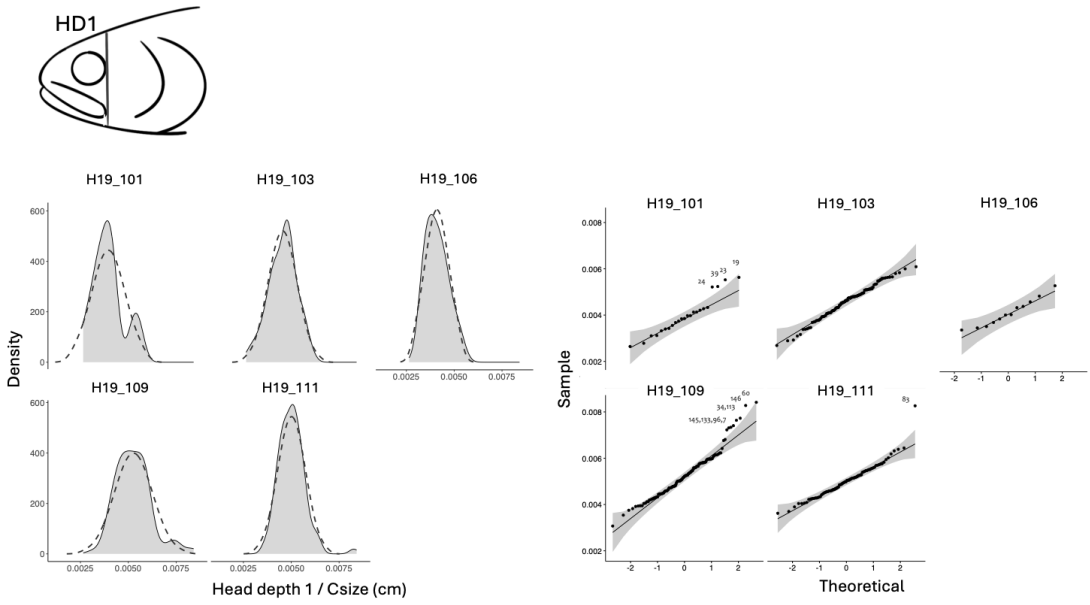
SM – Figure 5. Density distribution and Q-Q plots of *body depth* with outliers.



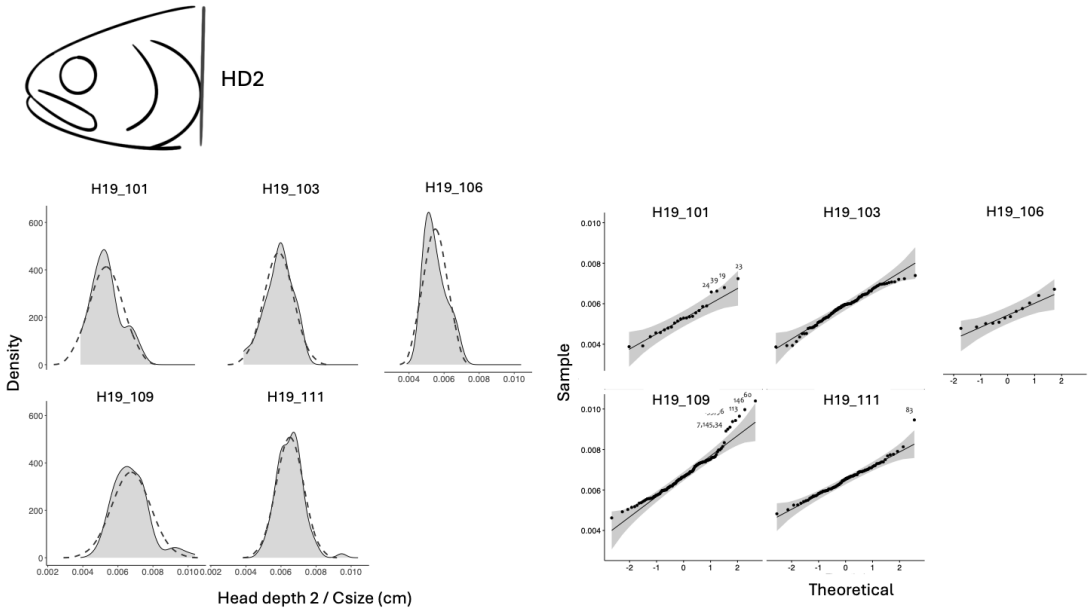
SM – Figure 6. Density distribution and Q-Q plots of *depth of dorsal fin* with outliers.



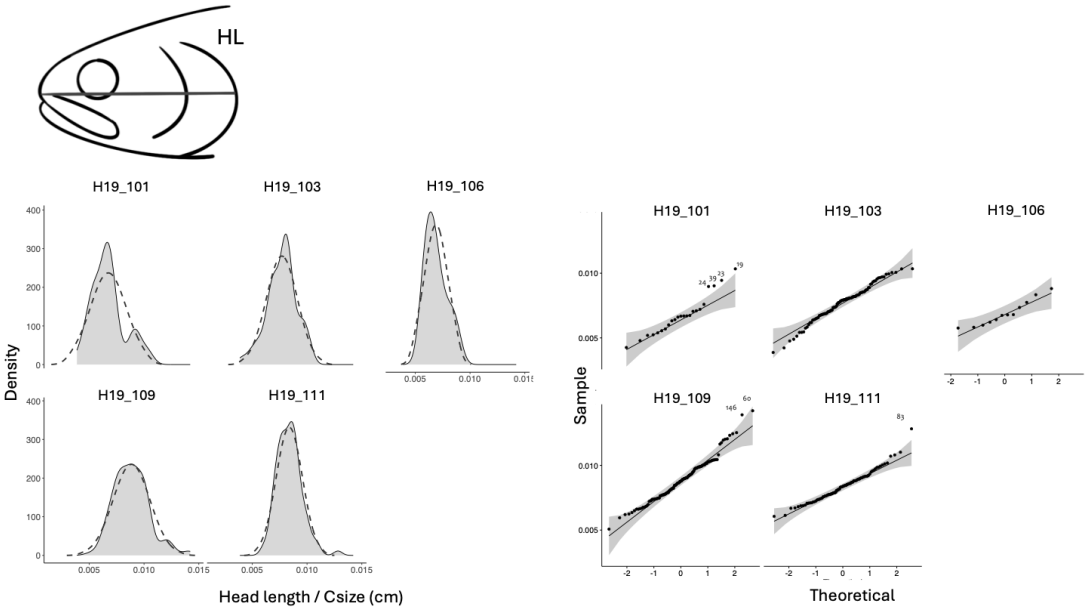
SM – Figure 7. Density distribution and Q-Q plots of *eye diameter* with outliers.



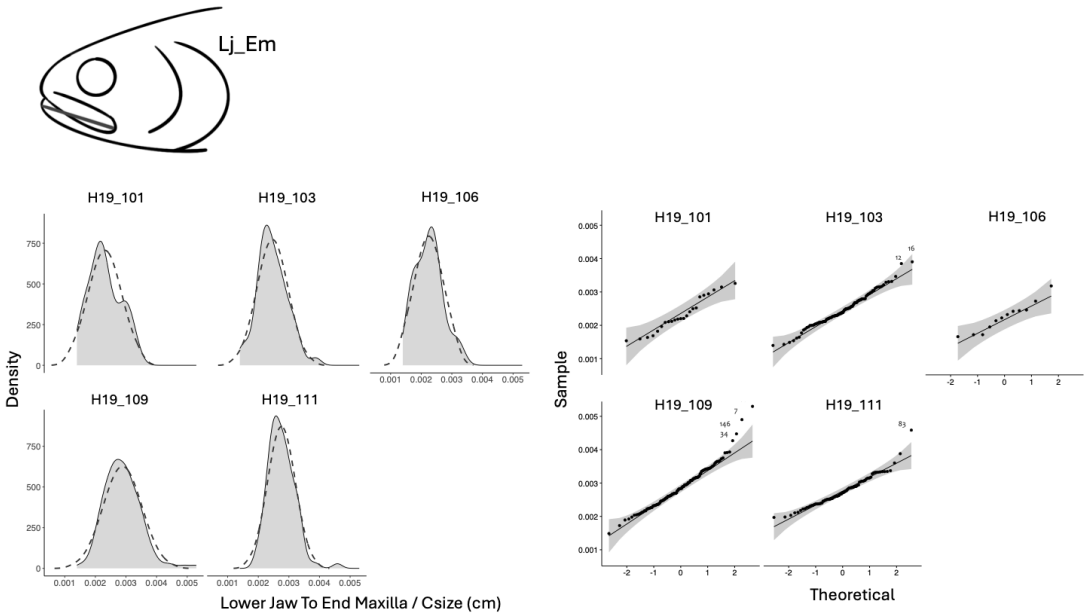
SM – Figure 8. Density distribution and Q-Q plots of *head depth 1* with outliers.



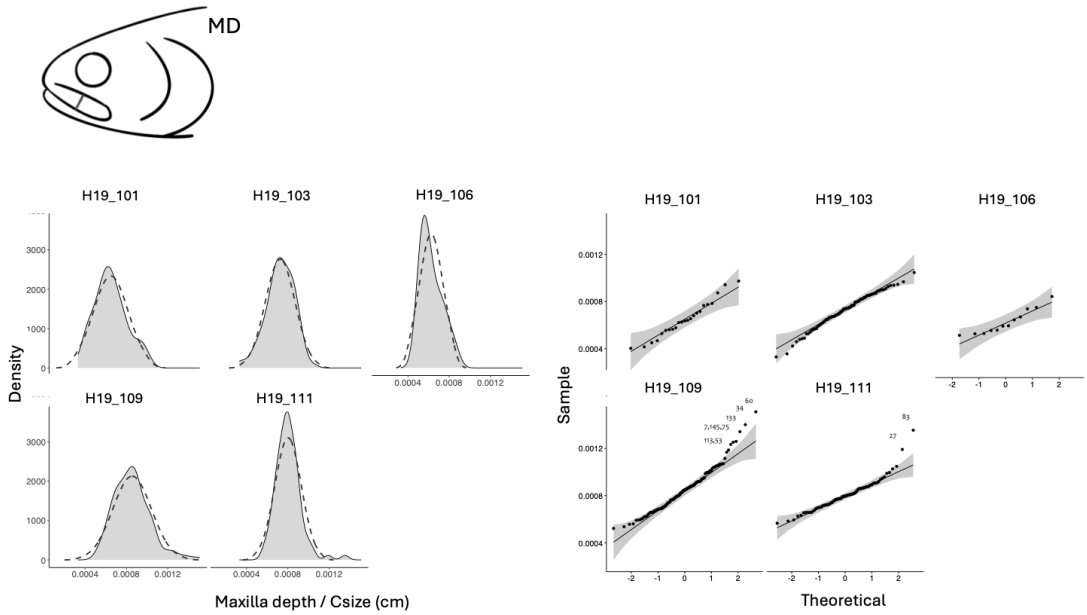
SM – Figure 9. Density distribution and Q-Q plots of *head depth 2* with outliers.



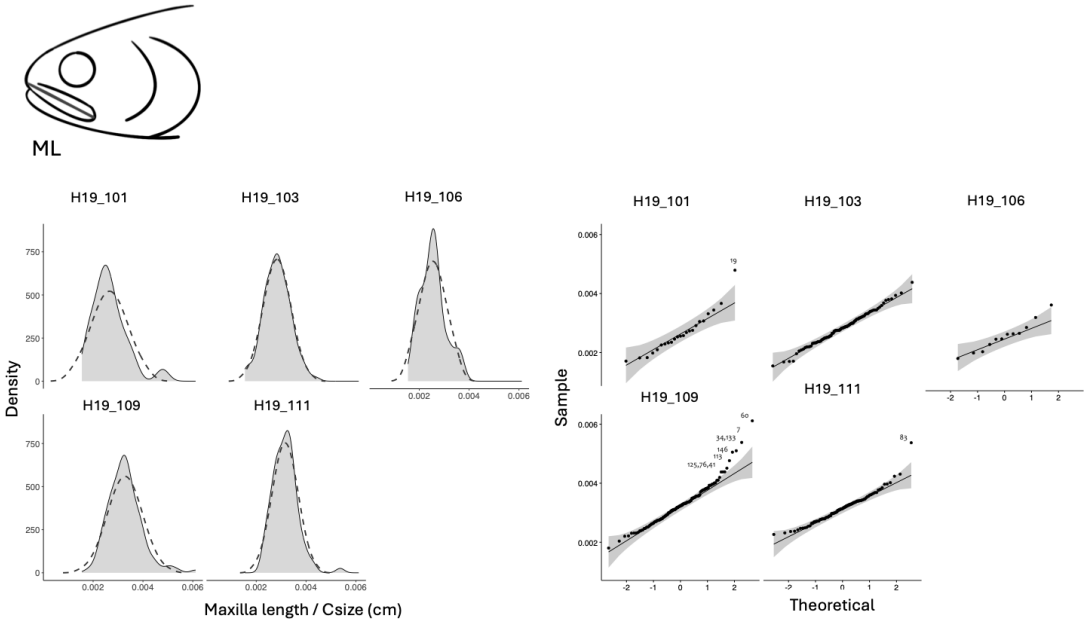
SM – Figure 10. Density distribution and Q-Q plots of *head length* with outliers.



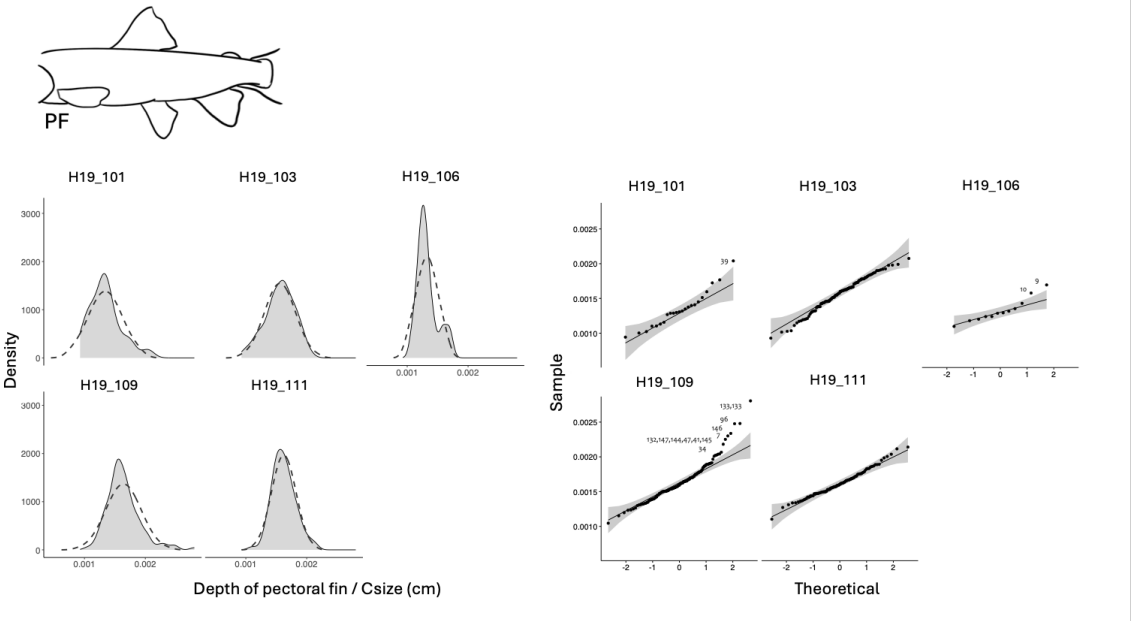
SM SM – Figure 11. Density distribution and Q-Q plots of *distance from the tip of the lower jaw to the end of the maxilla*, with outliers.



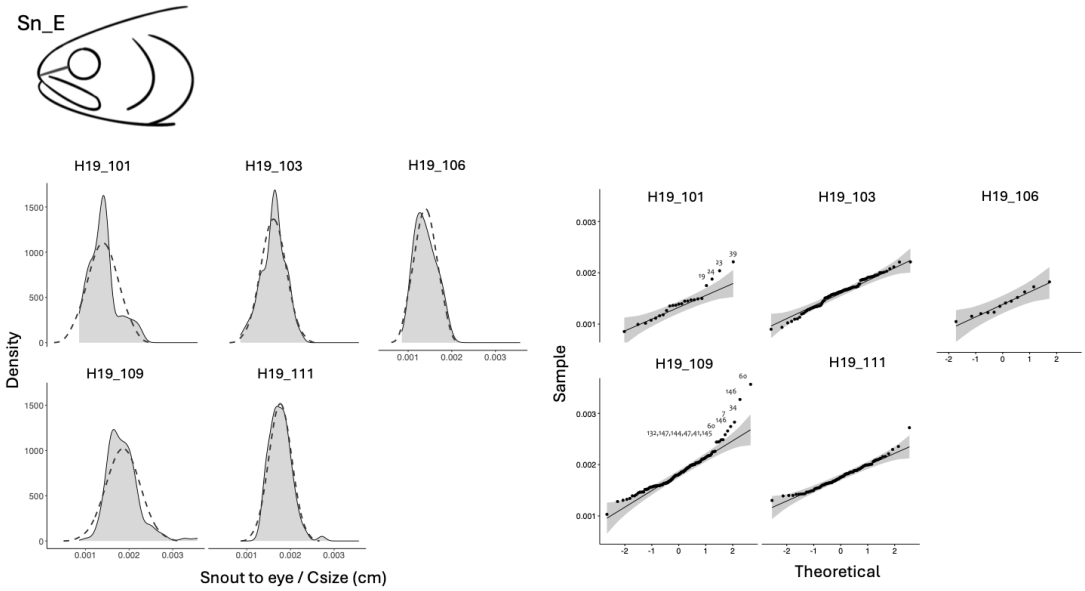
SM – Figure 12. Density distribution and Q-Q plots of *maxilla depth* with outliers.



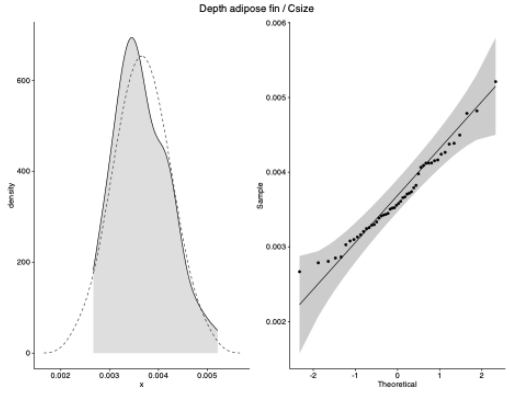
SM – Figure 13. Density distribution and Q-Q plots of *maxilla length* with outliers.

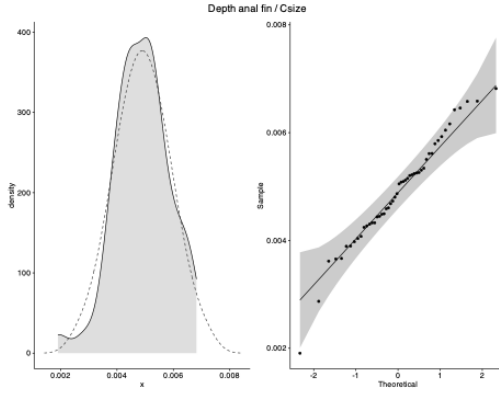


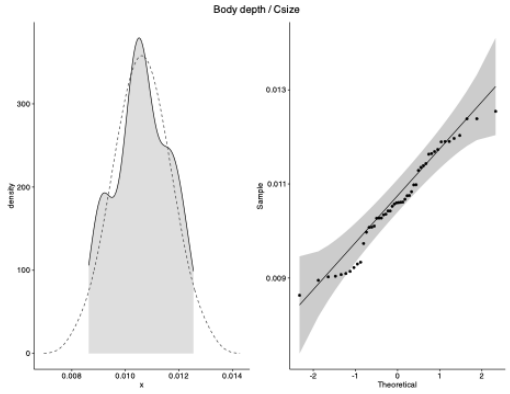
SM – Figure 14. Density distribution and Q-Q plots of *depth of pectoral fin* with outliers.

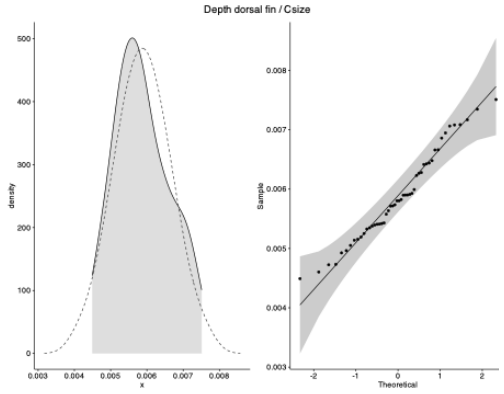


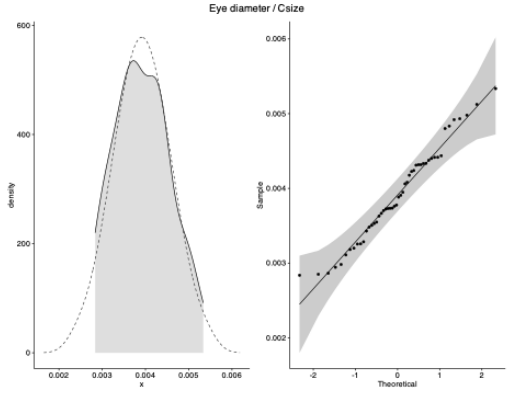
SM – Figure 15. Density distribution and Q-Q plots of *distance from snout to eye* with outliers.

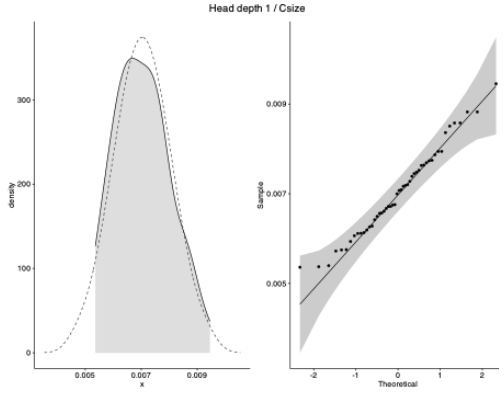

Depth adipose fin / Csize
density
x
Sample
Theoretical

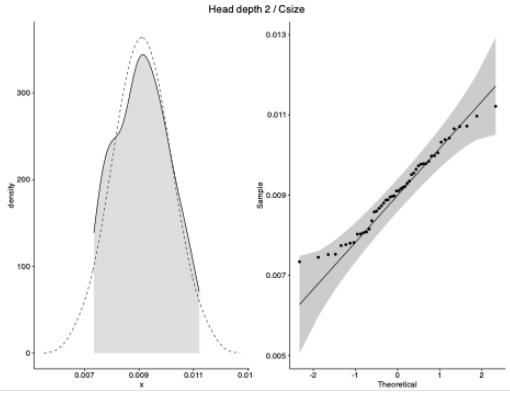

Depth anal fin / Csize
density
x
Sample
Theoretical

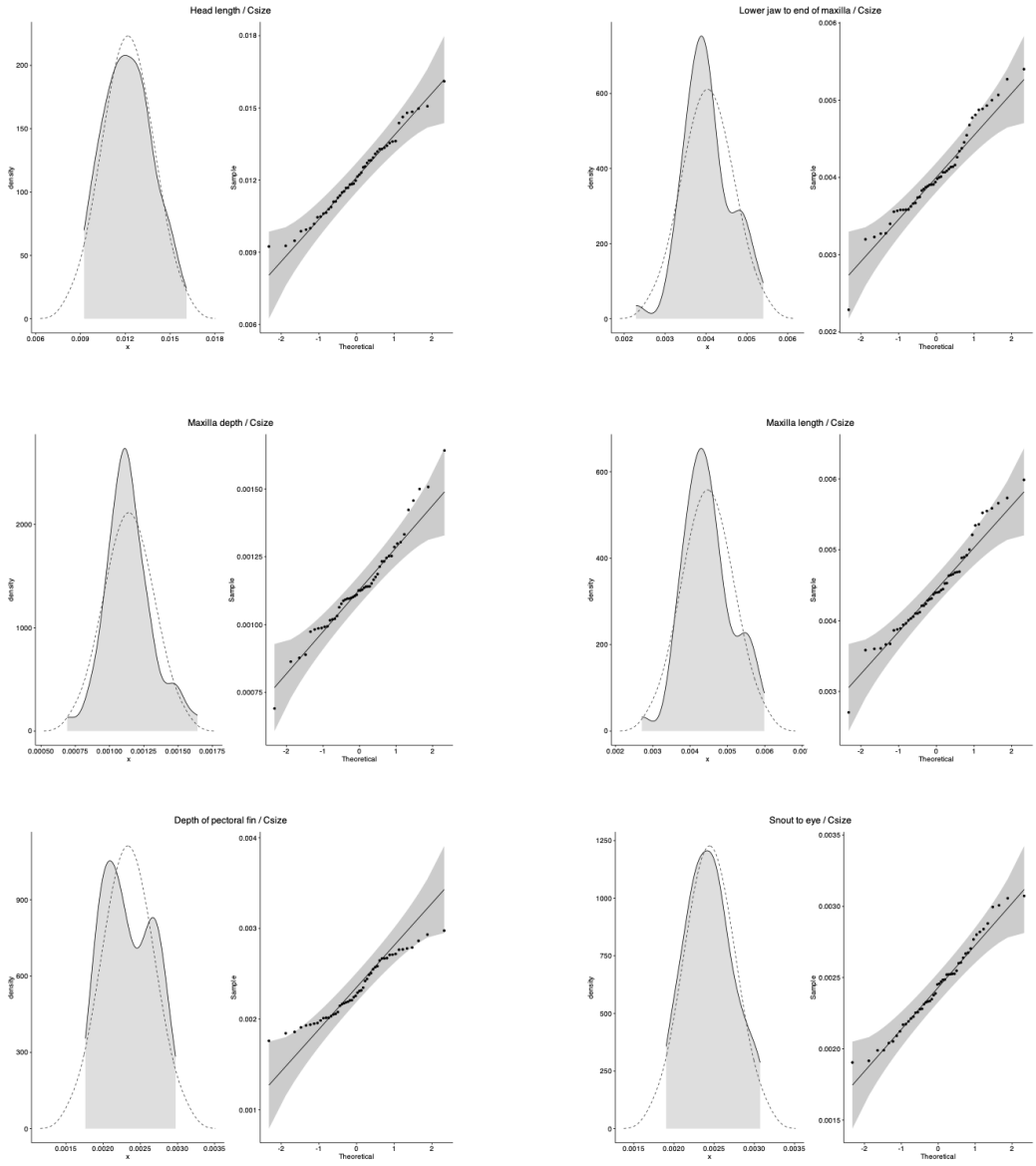

Body depth / Csize
density
x
Sample
Theoretical


Depth dorsal fin / Csize
density
x
Sample
Theoretical


Eye diameter / Csize
density
x
Sample
Theoretical


Head depth 1 / Csize
density
x
Sample
Theoretical


Head depth 2 / Csize
density
x
Sample
Theoretical



SM – Figure 16. Density distribution and Q-Q plots for all 13 linear measurements corrected by centroid size in the family H18-05.

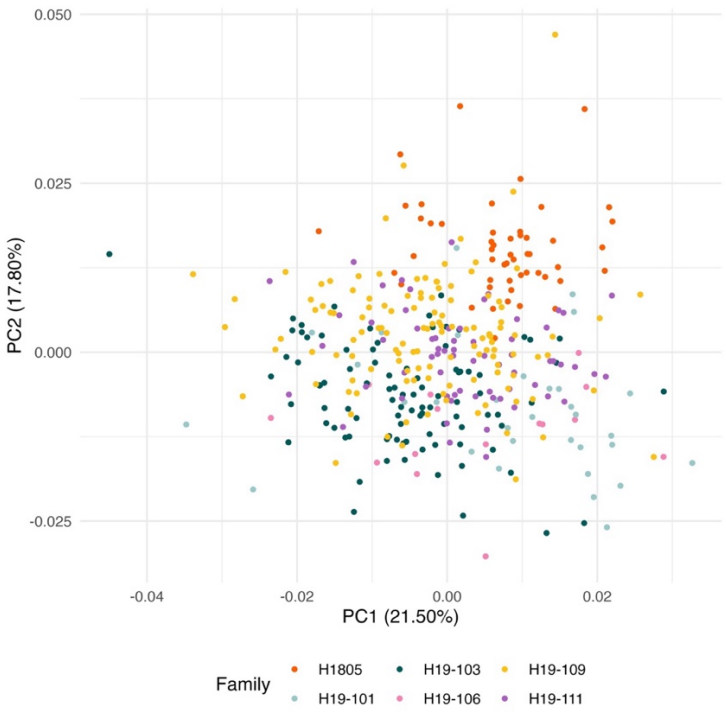
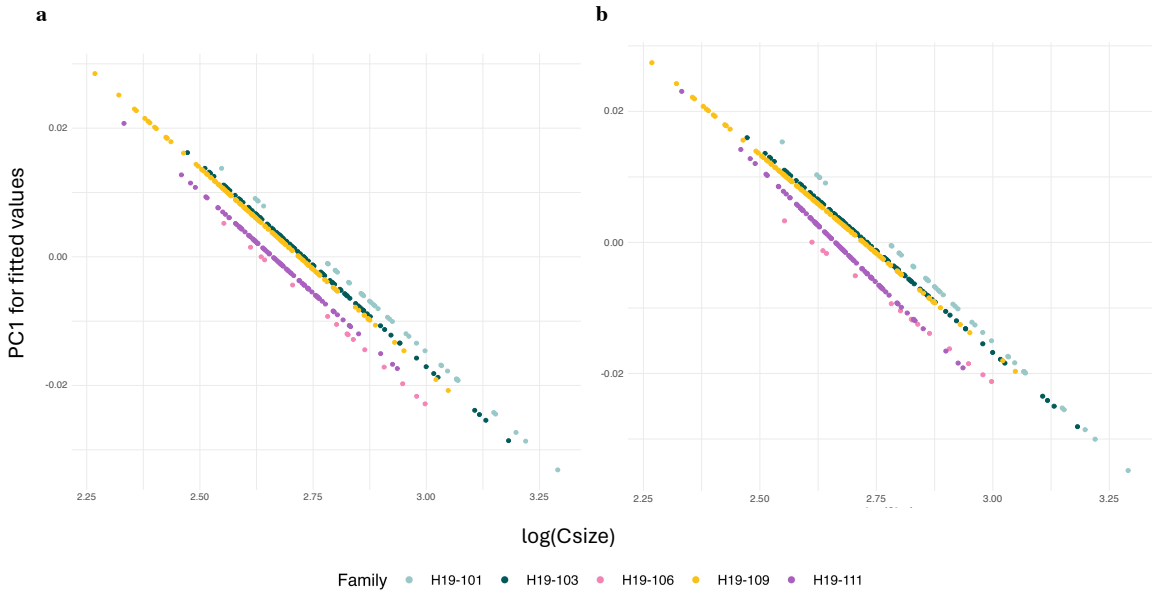
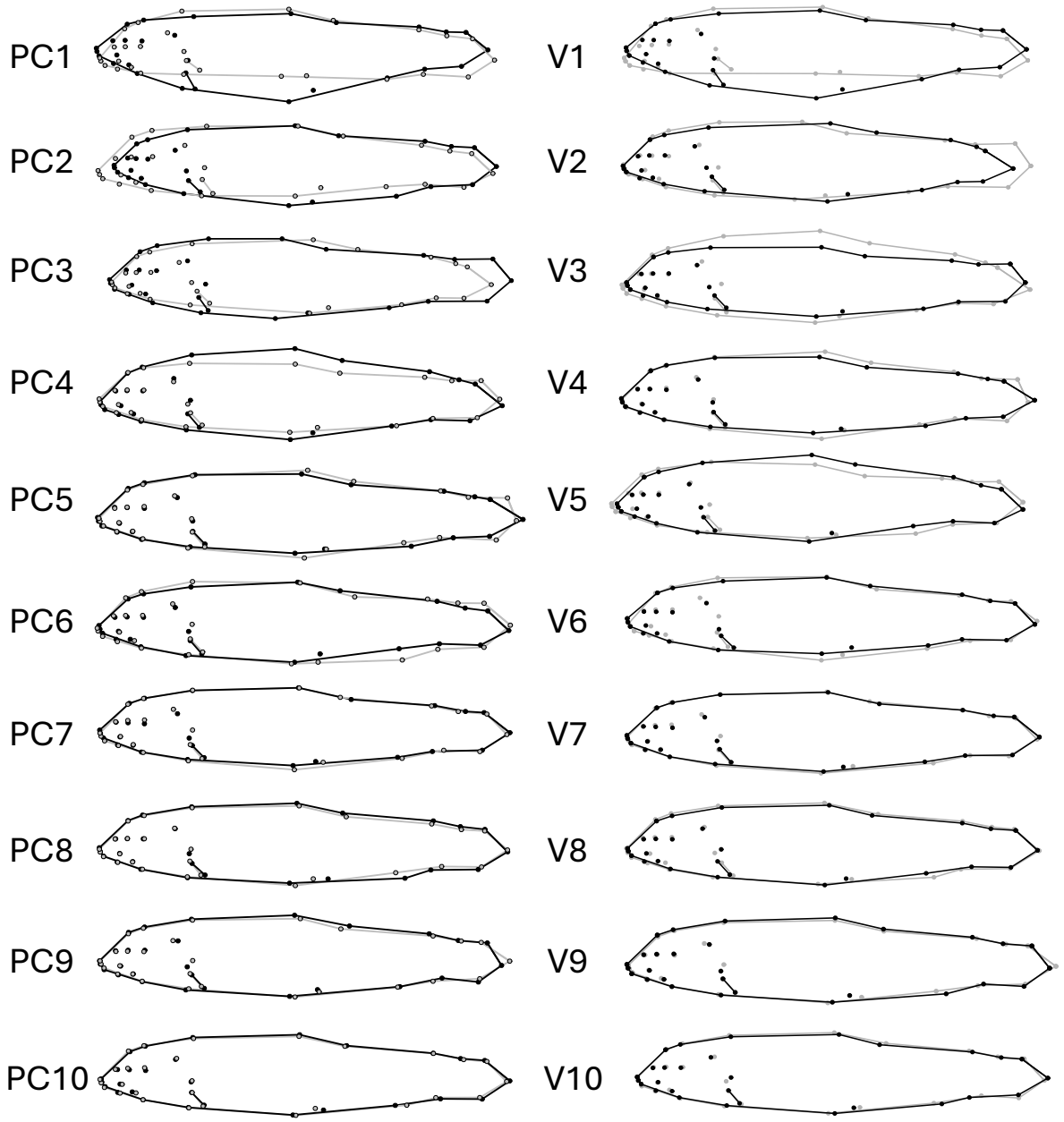


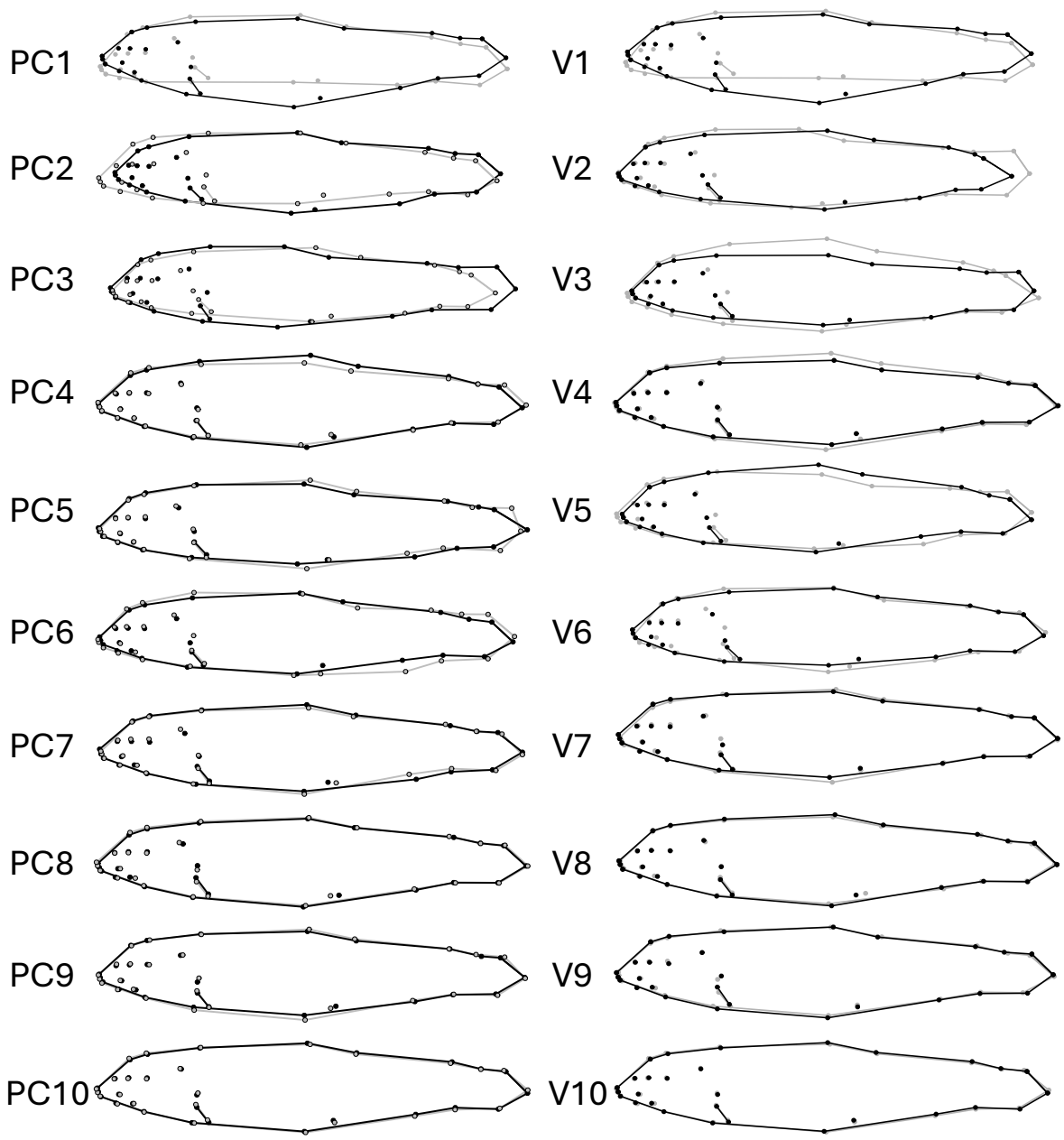
Figure 17. Principal Component plot of PC1 (21.50%) and PC2 (17.80%) from Procrustes landmark coordinates of the progeny of all six families. Each dot represents one individual, and different colorus represent the different families.



SM – Figure 18. Graphical representation of common allometric slopes (**a**) of all H19 families and unique allometric slopes (**b**) per H19 family for PC1 against log(Csize).



SM – Figure 19. Wireframes representing landmark configurations at the extreme of each PC axis from the PCA derived from Procrustes coordinates on the H19 families (PC1 – PC10) and from the PCA residuals derived from the simple allometry model (coords ~ log(Csize)) (V1 – V10). Grey wireframes represent the predicted shape at the lowest value of each eigenvector. Black wireframes rerpesent the predicted shape at the highest value of each eigenvector.



SM – Figure 20. Wireframes representing landmark configurations at the extreme of each PC axis from the PCA derived from Procrustes coordinates on the H1805 family (PC1 – PC10) and from the PCA residuals derived from the simple allometry model (coords ~ log(Csize)) (V1 – V10). Grey wireframes represent the predicted shape at the lowest value of each eigenvector. Black wireframes rerpesent the predicted shape at the highest value of each

PAPER III

Genetic processes involved in body size differentiation of the Arctic charr morphs from lake Thingvallavatn

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Abstract

Differentiation in body size is widespread in systems undergoing ecological specialisation. One such system is the Arctic charr in Thingvallavatn (Iceland), where, since the last glaciation, this species has evolved discrete large and small phenotypes resulting in two large morphs, a large benthic (LB) and a piscivorous (PI) charr, and two small morphs, a small benthic (SB) and a planktivorous (PL) charr. In this study, we investigate the historical and genetic nature of body size divergence. We demonstrate that the Arctic charr from Thingvallavatn comprises three genetic groups (SB, LB and PL/PI) and that the benthic and the limnetic morphs diverged early in the colonisation history of the lake. Additionally, we show that introgression can shape the level of genetic differentiation between morphs, mainly between the PL and the PI-charr. We then focussed on characterising highly differentiated regions in the genome shared between small and large morph pairs. One of these regions contains a gene (glypican-6) which is highly conserved throughout vertebrate evolutionary history. Glypican-6 is involved in cell proliferation and growth. During zebra fish development it has both maternal and zygotic expression. These data paint a complex picture where the genetic mechanisms behind body size differentiation are likely interwoven with phenotypic plasticity and maternal effects.

Keywords: population genetics, introgression, coalescence, glypican-6, Arctic charr

Introduction

Unveiling the origins of within- and between- population diversity is key to understand the processes leading to ecological specialisation. Briefly, ecological specialisation arises from standing phenotypic and genotypic variation through niche shifts, ecological sexual dimorphism and/or a combination of both (Araújo et al., 2011; Schoener, 1971) often leading to changes in the genetic structure of populations. Ecological specialisation usually occurs along predictable and well-established axes. For example, in the case of birds, fish and butterflies, populations (or even individuals within populations) can be either migratory or resident (Boyle, 2008; Chan, 2001; Chapman et al., 2011, 2012; Kerr et al., 2011; Malcolm et al., 2018; Slager & Malcolm, 2015), and in the case of aquatic organisms, populations can diverge along marine-freshwater or benthic-limnetic ecological axes (i.e., living near and feeding on the bottom as opposed to feeding in the water column (Jones et al., 2012; Skulason & Smith, 1995). Adaptations along these ecological axes often result in similar (or convergent) phenotypic outcomes. For instance, many benthic fish tend to have subterminal jaws and deeper bodies while limnetic fish have terminal jaws and more elongated bodies (Blake et al., 2005; Franchini et al., 2014; Friedman et al., 2020; Sandlund et al., 1992). Such convergent phenotypes may arise from standing genetic variation, selection and/or introgression (Fraser & Whiting, 2020) and can further be shaped by maternal effects and phenotypic plasticity (Crispo, 2008; Kristjánsson et al., 2018; Stein & Bell, 2019). However, the genetic and molecular mechanisms involved in the shaping of similar phenotypes usually differ (Elmer et al., 2014; Jacobs et al., 2020, Sailsbury et al., 2020, Fenton et al., 2024) but see (Jones et al., 2012).

Specialisation in a certain body size is a repeated phenotypic output in populations with high rates of diversification (Häkli et al., 2018; MacQueen et al., 2011; Perry et al., 2014; Roberge et al., 2006). It often manifests as a non-overlapping size distribution of sexually mature individuals. This size distribution can be sex-related, either between (Blanckenhorn & Blanckenhorn, 2005; Shine, 1989) or within sexes (e.g., guarding vs sneaker males (Gross, 1985; Sigurjónsdóttir & Gunnarsson, 1989; Young et al., 2013) or can be independent of sex but related to other factors such as migratory strategies (large resident and small migratory individuals (Boyle, 2008; Chapman et al., 2011; La Sorte et al., 2013), predation risk (large *L. saxatilis* crab ecotype vs small wave ecotype (Johannesson, 2003) and ontogenetic niche shifts (Claessen et al., 2000; Werner & Gilliam, 2003). For instance, a prominent example of ontogenetic niche shift in fish is the transition to piscivory. (Jensen et al., 2012; Sánchez-Hernández et al., 2019). Such diet shift requires the physical capability to capture fish, which is directly dependent on the individual's body size (Dörner & Wagner, 2003; Juanes, 1994). The mechanisms involved in the transition to piscivory and hence larger body size depend on the individual's genetic and physiological capabilities to grow and feed on larger prey, and on the ecological opportunities (i.e., the degrees of prey availability and competition) (Doenz et al., 2019; Gaeta et al., 2018; Keeley & Grant, 2001; L'Abée-Lund et al., 2002; Mittelbach & Persson, 2011; Nilsson et al., 2000). In size-structured fish populations, phenotypic plasticity and maternal effects can play a fundamental role in shifting towards higher trophic levels. Such shifts can additionally have a large impact in the community's dynamics (Hartvig et al., 2011; Sánchez-Hernández et al., 2019).

Evolutionarily young systems where species are undergoing ecological specialisation represent a unique opportunity to study the origins of repeated phenotypic specialisations. Although plenty of examples of such systems exist (Lee & Coop, 2019; Losos, 2011), sympatric systems which have repeatedly evolved along similar ecological axes are uncommon in nature. The Arctic charr (*Salvelinus alpinus*) in lake Thingvallavatn (Iceland) is one such system. Since the last glaciation this species has diverged along the benthic-limnetic ecological axis and has evolved discrete large and small phenotypes: a small benthic (SB) and a large benthic (LB) charr within the benthic morphotype and a small planktivorous (PL) and large piscivorous (PI) within the limnetic morphotype (Malmquist et al., 1985; Snorrason et al., 1989) (**Fig. 1**). Both benthic morphs feed on macroinvertebrates in the rocky littoral zone. While the SB charr utilizes interstitial spaces among lava rubble feeding mostly on the snail *Radix peregra*, the LB charr can, as they grow larger and less prone to predation, utilize the exposed part of the rocky epibenthos to take a variety of epibenthic invertebrates (Brachmann et al., 2020; Malmquist et al., 1992; Sandlund et al., 1987). Within the limnetic morphotype, the PL charr mainly feed on zooplankton and emerging chironomids in the open water column, whereas the juvenile PI charr, having grown to a certain size, switch to piscivory and feed almost exclusively on sticklebacks that are abundant among extensive stands of the green alga *Nitella opaca* (Brachmann et al., 2020; Kairesalo et al., 1992; Malmquist et al., 1992) (**Fig. 1**).

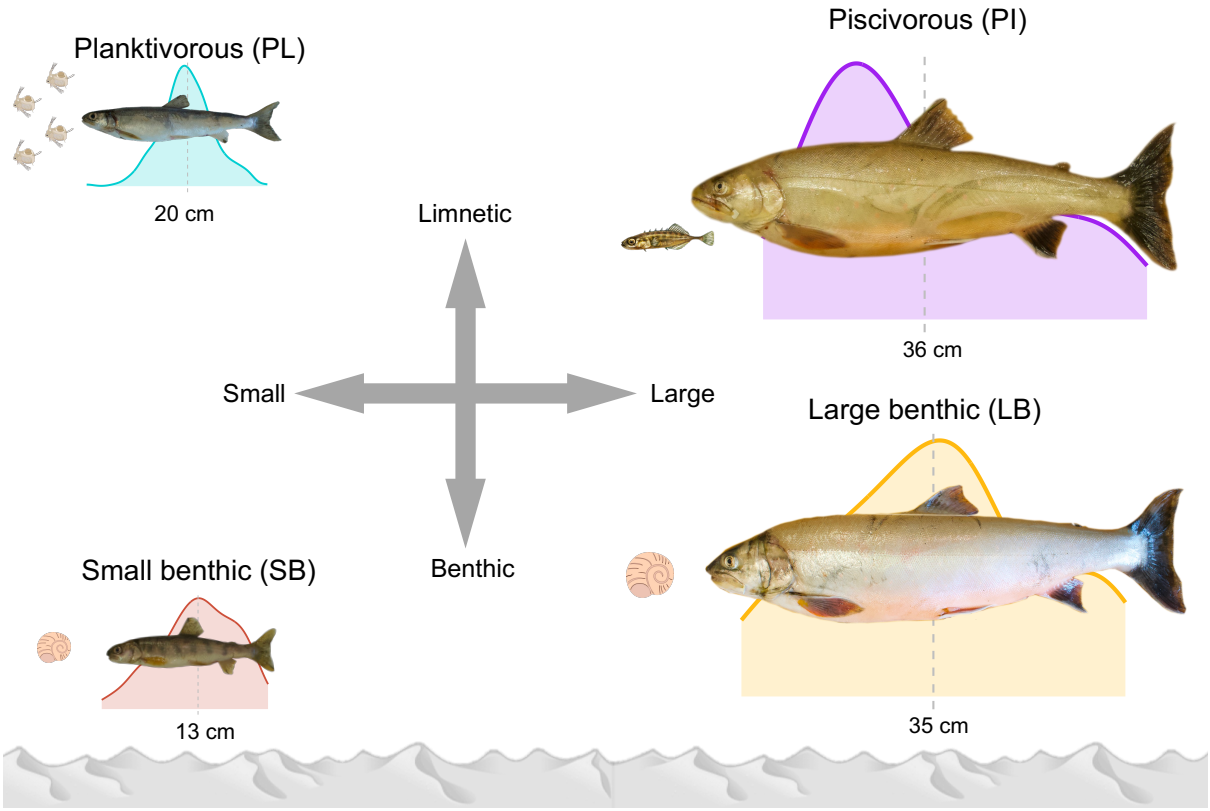

Figure 1. Diagram of the four morphs of Arctic charr in Thingvallavatn: shown are the benthic-limnetic ecological axis and the small-large body size specialisation, the main dietary preferences are illustrated on the left of each fish (not to scale). Density plots depict the fork length of the sampled specimens, and the dashed lines, the mean fork length.

The Arctic charr morphs in Thingvallavatn constitute a young polymorphic system presumably derived from invasions of anadromous fish during a single relatively short colonisation window that closed when unscalable waterfalls formed in the outflowing river (Ingólfsson et al., 1995). The colonising fish likely resembled the present-day anadromous charr in being a generalist feeder and maturing at a large size (Bengtsson et al., 2023). Coalescence simulations have pointed towards a rapid divergence of PL-, SB- and LB-charr probably involving microallopatry followed by secondary contact and sympatry Brachmann et al., 2022; Kapralova et al., 2011). At present these morphs represent three clear genetic clusters (Brachmann et al., 2020; Guðbrandsson et al., 2019). The understudied PI morph appears to be more enigmatic: the fact that it has not been possible to make a morphological distinction between juvenile PL- and PI-charr prompted the hypothesis that PI-charr were PL-charr that, possibly by chance, learned how to prey on sticklebacks, an ontogenetic niche shift that radically changed their growth pattern and life history (Malmquist et al., 1992; Snorrason et al., 1994). To some extent this has been corroborated by recent genetic studies but at the same time these data show clear signs of genetic connectivity between PI-charr and LB-charr (Guðbrandsson et al., 2019; Skúlason et al., 1989). Our limited understanding of the genetic origin and current genetic status of the PI-charr raises questions regarding its evolutionary trajectory within the broader context.

Given that it is likely that large adult body size and a PI-like morphology are ancestral characteristics (Bengtsson et al., 2023; Jonsson & Jonsson, 2005), it is safe to assume that the benthic morphology of the LB and SB charr and the smaller size of the PL and SB charr are derived characteristics which have presumably evolved via resource polymorphism (Malmquist et al., 1985; Snorrason et al., 1989, Malmquist et al., 1985; Snorrason et al., 1989). Irrespective of present genetic relationships, the evolutionary processes involved in the specialisation in certain adult body sizes across morphs are still unknown.

In this study, we investigate the historical and genetic processes of the body size specialisation observed in the Arctic charr system from Thingvallavatn. More specifically we ask the following questions: (Q1) what is the present population structure of the Arctic charr in the lake, when the PI morph is included? (Q2) how has gene flow between LB-charr and PI- affected the present genetic structure of the system? Based on these data we asked (Q3) what is the most parsimonious scenario of morph divergence in the Thingvallavatn system? And (Q4) what are regions of the genome that differ between the large and the small morphs? To address Q1 and Q2 we looked at the population structure and admixture patterns within the lake. To investigate Q3, we tested multiple evolutionary scenarios using backwards simulations to reconstruct the population history. Finally, to address Q4 we performed genome scans of small-large morph pairs from the lake.

Material and methods

Sampling of fish was conducted employing survey nets with 12 different mesh sizes (10, 12.5, 15, 18.5, 22, 25, 29, 33, 38, 43, 50, and 60 mm knot to knot) at three littoral transects, one in the northern part (Bjarnamöl) and two in the southern part (Miðfell, on the east coast and and Lambhagi on the west coast) (**SM – Fig. 1**). The nets were laid in the stony littoral zone (0-8m depth) at all three transects, in the Nitella zone (8-20m depth) at Miðfell and Lambhagi and in the profundal zone at 20-30m depth at Lambhagi. At Miðfell pelagic nets were also set in the water column above the Nitella zone. The fishing was done in late August and early September 2019 outside the main spawning season of the charr morphs.

Sexually mature fish were classified to morph following (Snorrason et al., 1989), their fork- length and weight measured and fin and muscle tissue samples taken for DNA extraction. DNA extraction was conducted on muscle or fin tissue using a phenol-chlorophorm protocol based on (Taggart et al., 1992). A custom ddRAD sequencing library preparation protocol was conducted following (Lagunas et al., 2023). A total of 275 samples (N_{PL} = 81, N_{PI} = 52, N_{SB} = 73 and N_{LB} = 69) distributed in four libraries (one library per lane) were sequenced with HiSeq X-ten at BGI HongKong. We increased the sample size of the PI morph compared to (Guðbrandsson et al., 2019), aiming to have comparable sample sizes across morphs.

We used a low representation, but genome-wide, sequencing method (ddRAD-seq) which account for SNP neutrality. Raw reads were demultiplexed using the *process_radtags* program in Stacks v.2.62 (Catchen et al., 2013; Rochette et al., 2019) and reads were truncated to 115bp. Reads were aligned to the Canadian *Salvelinus sp.* genome (Christensen et al., 2018, 2021) with the *bwa-mem2* algorithm (Md et al., 2019). *samtools* (Danecek et al., 2021) was used to generate and sort the individual‘s bamfiles, *gstacks* for SNP calling and *populations*, PLINK1.9 (Purcell & Chang www.cog-genomics.org/plink/1.9/; (Chang et al., 2015) and vcftools (Danecek et al., 2011) were used for filtering. A filtering step was applied to all raw variant calls where a maximum of 20% missing data was allowed per individual sample and locus. Variants with minor allele frequencies below 0.02 were subsequently removed. We then ran a second round of populations with the following labels: *-r 0.7 --max_obs_het 0.66 --write-single-snp* obtaining a final number of SNPs = 2932 and 229 individuals. Finally, we used *–-relatedness2* from vcftools to filter out individuals with a relatedness of third degree or higher, where only one individual was removed.

To characterise population structure we conducted PCA, AMOVA and ADMIXTURE. For this, we first performed linkage pruning with PLINK1.9 using *--indep.pairwse 10 3 0.2 (*i.e., window of 10kb, window step size of 10bp and pruning of variants with a $r^2 > 0.2$). To test the nested effects of size class (large vs small) and morph an AMOVA (Analysis of MOlecular VAriance) was conducted using the *poppr.anova* function in R/poppr (version 3.9.3, (Kamvar et al., 2014) with 100,000 permutations.

To reconstruct the divergence history, we conducted backwards simulations. For this the same pipeline was applied, this time merging PL and PI individuals into one population (i.e., PL/PI) due to their lack of genetic differentiation. The site frequency spectrum (SFS) was estimated using *easysfs* (https://github.com/isaacovercast/easySFS; (Gutenkunst et al., 2009). Eight coalescence scenarios were tested (**Fig. 3A-3D**) using fastsimcoal2 (Excoffier

et al., 2013, 2021). We compared scenarios where (1) PL/PI were assumed to be ancestral and all three populations diverged at the same time (explosive radiation **Fig. 3A**), (2) where PL/PI were assumed to be ancestral and SB and LB diverge later in the population history (PL/PI ancestral, **Fig. 3B**), (3) where PL/PI evolved more recently and LB is the ancestral population (LB ancestral, **Fig. 3C**) and (4) PL/PI evolved more recently and SB is the ancestral population (SB ancestral, **Fig. 3D**). All four models were run with and without migration. In models 2-4, migration starts among all morphs right after the second divergence event. In model 1, migration starts at T_{MIG}=unif(1,3000), but see a description of the parameters in **SM 9**. We conducted *fsc2709* (with options *-M -m -n 200000 -L 60 -s 0 -x -c 12 –q*) for 50 independent runs per model to find the best parameter estimates. Within each model, the best run was selected, and the AIC calculated, as well as the likelihood distributions for model comparison (N_{perm}=10000). For the most likely model, parameter estimates were checked by bootstrapping the variant sites, resulting in 50 random bootstrap blocks. The most likely model was re-run 100 times for each bootstrap block and the best run was selected. We then bootstrapped the mean and the standard deviation (10,000 repetitions) of all the parameters to obtain 95% confidence intervals. We additionally tested models where PL- and PI-charr were treated as distinct genetic populations, given that potential complex hybridisation patterns among LB-, PI- and PL-charr could explain the lack of genetic differentiation between PI- and PL-charr (described in **SM Fig. 2**).

To identify genomic regions related to differences in life history traits, especially size at maturity, per-site F_{ST} values between all small–large morph pairs (PI-PL, PI-SB, LB-PL and LB-SB) were calculated. Markers belonging to the highest 97.5 quantile were kept for subsequent analyses. Thus the selected threshold for each morph pair differed, accounting for heterogeneity in evolutionary histories. Shared F_{ST} outliers were visualised with a 4D Venn diagram (Gao et al., 2021). The F_{ST} outliers shared by trios or duos of small-large morph pairs were further investigated using the Salvelinus spp. annotated genome (Christensen et al., 2018, 2021). We acknowledge that ddRAD sequencing may not be ideal to conduct genome scans (Lowry et al., 2016), thus we are exercising caution by 1) mostly focusing on SNPs belonging to functional parts of the genome and 2) being conservative in claiming signatures of selection when detecting a signal (Lowry et al., 2017). Furthermore, ddRAD-seq has been shown to be an effective tool to explore population genetics and conduct genome scans in non-model organisms with large and complex genomes (Catchen et al., 2017; McKinney et al., 2016), such as the Salvelinus spp. (~2.4 billion nucleotides) (Christensen et al., 2018). We also investigated whether relevant F_{ST} outliers contained miRNA target sites by using the Atlantic salmon (*Salmo salar*) miRNA database (Bekaert et al., 2013; Skaftnesmo et al., 2017) from miRbase (Griffiths-Jones, 2004; Kozomara et al., 2019) using the software miRanda v3.3a (Enright et al., 2003).

Results

Three genetic clusters: LB, SB and PL-PI, with signatures of introgression between LB and PL-PI

The Principal Component Analysis (PCA) of the genomic data showed genetic clustering of individuals by morph in the first two principal components. Individuals grouped in three genetic clusters: cluster 1 consisting of SB charr, cluster 2 mainly consisting of LB charr and cluster 3, comprising the PI and PL charr (**Fig. 2A**). Benthic and limnetic morphs differed along PC1 (7.91%). The small and large benthic morphs diverged along PC2 (3.33%), and a consistent but weaker trend was seen in the limnetic morphs. A large proportion (59.5%) of fish classified as PI-charr clustered within the 95% ellipse of PL charr. Of the 17 remaining individuals, 11 (26.2%) formed their own subcluster, two (4.8%) clustered with LB-charr and four (9.5%) spanned the space between the two. This suggests a complex and admixed genetic structure of PI-charr (**Fig. 2B**). No clear divergence of PL- and PI-charr could be discerned in the first 10 PCs (cumulative variance = 19.88%). We saw signatures of population substructure within the limnetic cluster along PC3 (1.67%) (**SM – Fig. 3**). Congruently with the PCA, we found significant differences in population F_{ST} between all morph pairs except for PL and PI (**SM – Table 1**). The AMOVA revealed that while there was no genetic variance attributed to size the effect of morph within size was highly significant ($p < 0.001$) and accounted for 12.5% of the genetic variance (**SM – Table 2**).

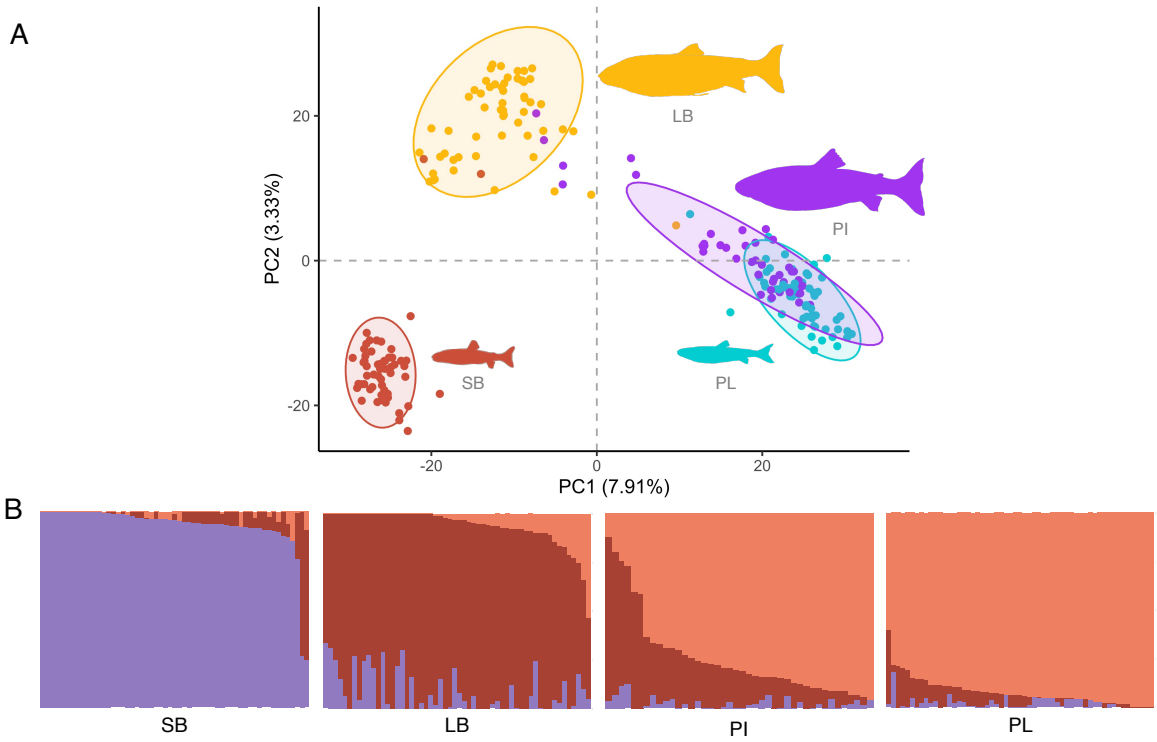

Figure 2. (**A**) Principal Component Analysis (PCA) plots of 228 individuals (55 SB-, 59 LB-, 42 PI- and 72 PL-charr) and 2932 SNPs. Each point represents an individual, and 95% confidence ellipses are shown for each morph. (**B**) ADMIXTURE plot from SNP data

for K=3 from 212 genotypes. Each colour represents an ancestry cluster and individuals are ordered by their Q values.

The results from the ADMIXTURE analysis yielded the same population structure seen in the PCA and further explained the introgression patterns within the lake (**Fig. 2B**), with the caveat that other processes such as incomplete lineage sorting might be at place. The number of K clusters K=3 and K=4 had the lowest cross-validation error (**SM – Fig. 4**). For K=3, the four morphs show different levels of genetic mixing: the PL- and SB-charr were more genetically homogeneous, whereas the genetic structure of the large morphs (LB- and PI-charr) was more complex. This is most clearly seen in the PI-charr where a high proportion of individuals showed high levels of introgression with LB charr (**Fig. 2B**). When K=4, our results reflected the subpopulation structure observed in PC3 (**SM – Fig. 3**) (**SM – Fig. 5**).

The PL/PI genetic cluster has ancestral origins

We reconstructed the population histories of the three genetic populations of Arctic charr in Thingvallavatn using coalescence simulations. Of the eight tested models (**Fig. 3A**), one was found the be the most likely: the model assuming ancestry of the PL/PI population with the LB- and SB-charr diverging later and subsequent moderate geneflow among all populations (PLPIancMig (**Fig. 3B, SM – Table 3 and 4**). The coalescence simulations thus lent support to the hypothesis that a limnetic (PI/PL) ancestor, likely resembling present anadromous charr in morphology and adult size, first colonised the lake and the two benthic morphs diverged from that branch (**SM-9**).

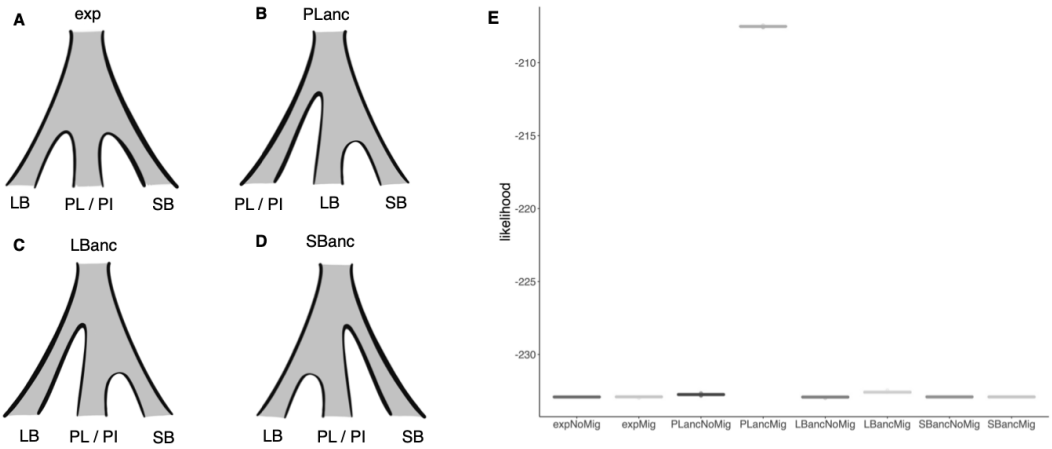

Figure 3. (**A**) Tested divergence scenarios of the three distinct genetic groups of Arctic charr in Thingvallavatn. Each model was tested without and with recent constant geneflow, not depicted. A = exp, B = PLPIanc, C = LBanc, D = SBanc. (**B**) Boxplot of likelihood distributions of the eight tested coalescence models. The best supported model within each population had the greatest maximum likelihood value. The boxplot whiskers represent 95% confidence intervals for each model.

We also ran models where the PL and the PI charr were considered separate populations. Both AIC values and likelihood distributions from these 10 models pointed towards the model LBPImig being the most likely (**SM – Fig. 6**, **SM – Table 5**). In this model, SB, LB and PL charr diverge at the same time in the population history and PI diverges more recently from LB charr. The benthic-limnetic divergence (ben.limMig) and admixture with migration (admMig) models were the second and third most likely, respectively.

Different genomic regions are involved in divergent adult body sizes between the benthic and limnetic morphs

To investigate the genetic factors related to body size at maturity we first performed genome scans contrasting large and small morph pairs (**Fig. 4**). Because of their contrasting body sizes and similar modes of feeding, we focussed on PI-PL and LB-SB F_{ST} outliers (**Fig. 4A, 4B**, **SM Table 6, SM Table 7**). While the F_{ST} outliers of both limnetic and benthic contrasts were located in isolated regions of the genome, the F_{ST} outliers in the PL-PI contrast differed markedly from the those seen in the LB-SB contrast regarding location, distribution (**Fig. 4A and 4B and SM Table 6** and **7**) and proportion of shared outliers with other large-small pairs (**Fig. 4C**).

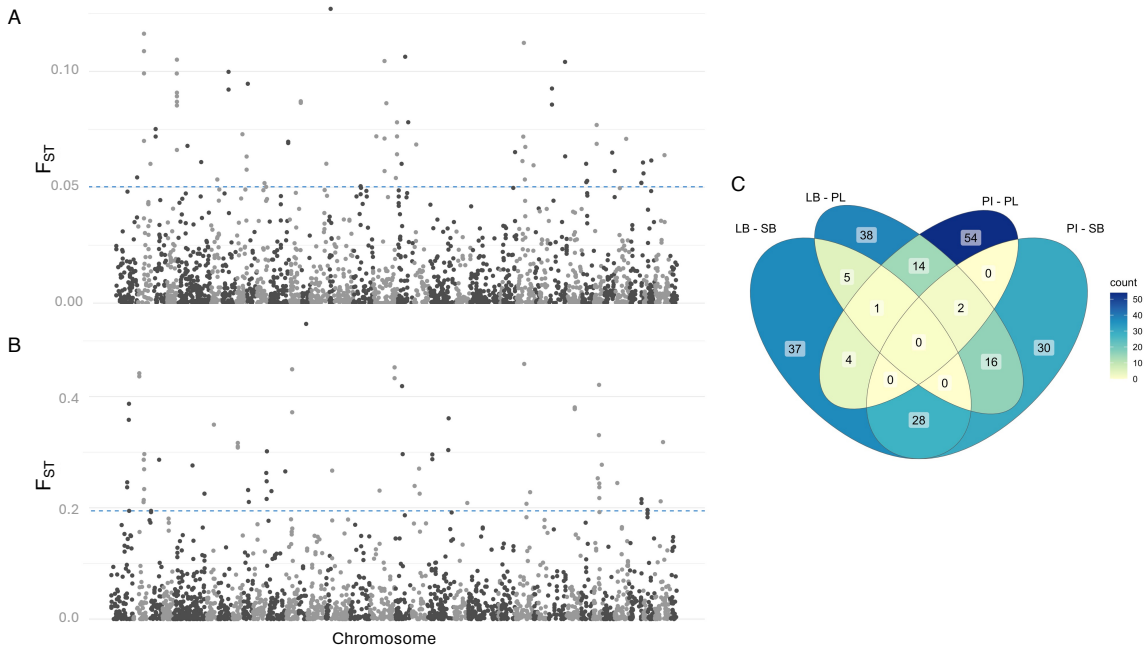

Figure 4. F_{ST} outlier analysis. (**A**) Manhattan plot of F_{ST} outliers between PL and PI and (**B**) between LB and SB. For both A and B, the blue dashed line indicates the 97.5% threshold for each morph pair. Note different in scale for A and B. Alternating gray and black colours refers to separate chromosomes. (**C**) Venn diagram showing the number of shared and unique outliers among all four large-small morph pairs.

Next, we looked at shared F_{ST} outliers among all small-large morph pairs from the lake (i.e., LB-SB, LB-PL, PI-PL and PI-SB) (**Fig. 4C**). No outliers were shared among all four

small-large morph pairs, and while very few outliers (3 SNPS) were shared among three of the pairs, a larger proportion of outliers (63 SNPs, 26.5%) was shared between two of the morph pairs (**SM – Table 8**). There were three F_{ST} outliers shared by three morph pairs, one belonging to LG16 and two to LG26. One of these outliers which was shared among LB-PL, PI-PL and PI-SB is located on LG26 within a coding region related to translation repression. The remaining two SNPs were found at least ±10,000bp away from any known coding regions (**SM – Table 8**). The 66 outliers shared between large-small pairs were not uniformly distributed throughout the genome: the highest number of shared outliers being on LG2 (12 SNPs, localised in 8 different regions) and on LG30 (6 SNPs in 6 different regions) **SM – Table 8**). Because of their contrasting body sizes and similar modes of feeding, and to avoid those SNPs potentially associated to adaptations to benthic-limnetic lifestyles (i.e., LB-PL and SB-PI), we focussed on PI-PL and LB-SB F_{ST} outliers (**Fig. 4A, 4B, SM Table 6, SM Table 7**).The F_{ST} outliers of both limnetic and benthic contrasts were sparsely located in isolated regions of the genome, differing markedly regarding location, distribution (**Fig. 4A** and **4B** and **SM Table 6 and 7**) and proportion of shared outliers with other large-small pairs (**Fig. 4C**). Only 4 outlier SNPs were shared between the small-large morph pairs within their benthic or limnetic morphotype. One SNP was located in a pseudogene and three SNPs were in a coding region for the gene *glypican-6*, involved in growth and cell division (Gupta & Brand, 2013). Two *gpc-6* outliers, one shared in the PL-PI and SB-LB contrasts, and the other one shared between PL-LB and PL-PI, formed a haplotype of two SNPs separated by 6bp and located in the 3'-untranslated region (3'-UTR) of the gene (**Fig. 5**). Because the 3'-UTR is known for containing binding sites for post-transcriptional regulators, we scanned for miRNA target sites spanning the haplotype, using the Atlantic salmon miRNA database (Bekaert et al., 2013; Skaftnesmo et al., 2017). While a total of 5 miRNAs targeted this region (miR-29c-5p, miR-365-5p, miR-27d-5p, miR-106a-3p and miR-130d-1-5p), two of them differed among haplotypes: miR-29c-5p (for SNP_1=A and SNP_2=T), and miR-130d-1-5p (for SNP_1=C and SNP_2=G, (**Fig. 5**). The last SNP belonging to *gpc-6* was located in an intron.

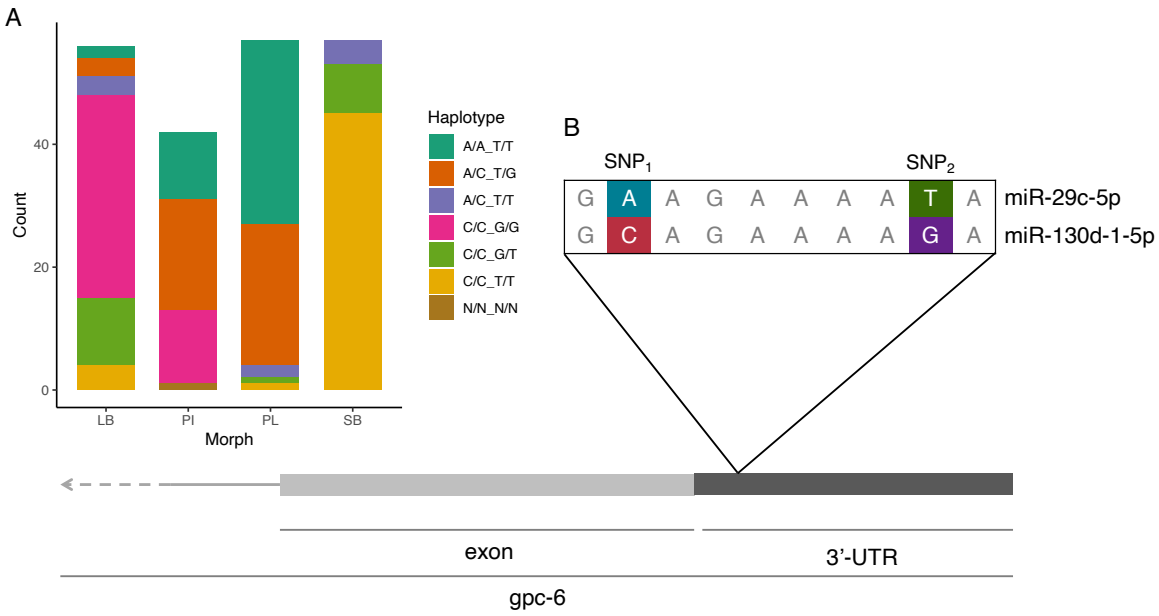

Figure 5. (A) Frequency of *gpc6* 3'UTR haplotypes by morph. The first term of the legend represents the possible alleles for SNP_1 and SNP_2 , separated by a slash. **(B)** Diagram of the of the last exon (»150bp) and 3'-UTR (»400bp) region of the gene *glypican-6* (*gpc6*, total length =117,201 bp). The sequences above represent the two haplotypes targeted by different miRNAs: miR-29c-5p (for SNP_1=A and SNP_2=T, depicted in dark green for homozygotes and orange and purple for the different combinations of heterozygotes) and miR-130d-1-5p (for SNP_1=C and SNP_2=G, depicted in pink when homozygotes and light green in heterozygotes).

Discussion

Our results indicated that the four sympatric Arctic charr morphs from Thingvallavatn are comprised of three genetic groups, with the piscivorous morph having an admixed genetic makeup. Despite the genetic similarities between the PL and the PI morphs, it is a higher level of introgression with the LBs that influence the genetic makeup of the PI morph. Coalescence simulations pointed towards the benthic and the limnetic morphs diverging early in the colonisation history of the lake. These results build a promising framework to study the genetic underpinnings involved in the vastly different life history characteristics of these charr morphs. We found highly differentiated regions in the genome shared between small and large morph pairs, where a few F_{ST} outliers were located in coding regions with known function. One of these regions contained a gene (*glypican-6*), which is highly conserved throughout vertebrate evolutionary history and is involved in cell proliferation and growth.

The genomic data confirm previous studies about the population structure of Arctic charr in the lake (Brachmann et al., 2021; Guðbrandsson et al., 2019; Kapralova et al., 2011), but also provide new insights. The SB- LB- and PL-charr formed three clusters along the first two PC axes. The genetic structure of PI-charr was more complex. Although the PI- charr had a very similar genetic makeup to PL-charr, the signatures of introgression from the LB-charr were clear, and individual genomes varied along a gradient from being purely PL-like to being LB-like. On one hand these data corroborate the hypothesis that PI-charr are PL-charr that go through an ontogenetic niche shift to piscivory (Malmquist et al., 1985, 1992; Snorrason et al., 1994). On the other hand, it is clear that the PL/PI-charr are and have been mixing with LB-charr and this genomic input may have conferred some PL/PI individuals with the ability of growing larger and consequently shifting their resource use towards piscivory. Furthermore, we found that many fish categorized as PI-charr can have both LB- and PL-like genomes, which emphasizes the potential of environmental conditions triggering ontogenetic niche shifts via phenotypic plasticity (Parsons et al., 2011). The substructure seen in the PL/PI genetic cluster (K=4 in the admixture analyses, **SM Figure 3**) may indicate i) strong spawning site fidelity of the limnetic morphs, suggesting that the transition to piscivory may be ubiquitous in lake (Guðbrandsson et al., 2019; Malmquist et al., 1992; Skúlason et al., 1989; Snorrason et al., 1989) or ii) reflect genetic contribution from another (or ghost) population to these two morphs in the lake. However, the stability of recruitment and the generational admixture

patterns of the PI morph remain unknown, as most specimens were sampled in their feeding grounds rather than in their spawning grounds. The patterns indicating introgression in the data land credence to the ongoing hybridisation between the LB and the PI morphs in the lake (Guðbrandsson et al., 2019). For this to happen, we might predict a lack of strong pre- and post-zygotic barriers between these morphs (Jonsson et al., 1988). Interestingly, this is not the case for other pairs of morphs within Thingvallavatn (for instance the LB morph is reproductively isolated from the rest by its early spawning time (Skúlason et al., 1989), and complex patterns of reproductive isolation have been studied between the SB and PL morphs (Horta-Lacueva et al., 2021).

The reconstruction of the evolutionary histories of the three genetic Arctic charr populations supported a scenario of two divergence events, separating the limnetic and the benthic morphs early in the colonisation history, the LB and SB populations diverging later. Earlier studies including the LB, SB and PL charr pointed towards a scenario of rapid and explosive divergence of LB, SB and PL with geneflow among all populations after an allopatry period (Brachmann et al., 2022; Kapralova et al., 2011). Setting the genetically quasi-identical PL and PI morphs as separate populations in coalescence modelling was conceptually challenging, as one would expect the PL and PI populations to coalesce fast, however the evident (and likely asymmetrical) geneflow from LB would make other scenarios likely. Our result (the PI from LB model) emphasizes that introgression between LB and the PL/PI population play an important role in defining the genetic status of the PI charr.

When focussing on the regions of the genome that were highly differentiated between the PL and PI morphs, we found that two of these were located in the coding region of the *gpc6* gene. Interestingly, *gpc6* is involved in cell proliferation and growth (Gupta & Brand, 2013b), and is highly conserved throughout vertebrate evolutionary history (Campos-Xavier et al., 2009; Capurro et al., 2017; Gupta & Brand, 2013b; Shi et al., 2020; Veugelers et al., 1999). One of these differentiated regions in *gpc6* was also shared between the SB and the LB morphs, suggesting that the same regions of the genome may be at least partially responsible for the parallel body size differences seen between the limnetic and benthic morph pairs in the lake. These genetic islands of differentiation may have arisen due to differential allelic variation responding to divergent selection, which was maintained and transferred to other morphs via introgression. Although not mutually exclusive, it may be the case that the genetic islands of differentiation explaining body size diversification may have arisen from ancestral standing genetic variation and selected independently after the split of the benthic and limnetic branches. Moreover, the “islands of differentiation” hypothesis is not exclusive with a genome-wide differentiation theory, seen in the literature about ecomorph divergence in charr (Guðbrandsson et al., 2019; Kess et al., 2020). The relatively recent divergence of the morphs and the potentially complex patterns of introgression accentuate the difficulties of distinguishing parallel evolution from parallel standing genetic variation, especially in a system where evolution through adaptive introgression and selection on standing variation may not be mutually exclusive (Bassham et al., 2018; Fraser & Whiting, 2020; Lee & Coop, 2019; Taylor et al., 2020).

Gpc-6 is involved in the control of cell growth and cell division and in fish it is a putative marker for maternal effects (Capurro et al., 2017; Gupta & Brand, 2013; Shi et al., 2020; Veugelers et al., 1999). In zebrafish, two *gpc6* isoforms have been described (*gpc6*a and *gpc6*b) which show ubiquitous zygotic and maternal expression throughout early development. In later stages (during segmentation) *gpc6a* is expressed in the developing

gut while *gpc6b* is expressed in the yolk. The fact that the *gpc6b* expression has been detected in the yolk indicates that maternal mRNA deposited in the egg may be influencing development (Adrian-Kalchhauser et al., 2020) long after the maternal to zygotic transition has taken place (Lee et al., 2014, Chang et al., 2018). Candidate genes such as *gpc6* are of particular interest given that, in fish, maternal effects play an important role on phenotypic plasticity, life history traits, niche construction, response to selection and population dynamics (Beckerman et al., 2002; Benton et al., 2005; Wolf & Wade, 2009). In wild populations of Icelandic Arctic charr, maternal effects have been shown to impact egg size, juvenile body size, clutch size, social behaviour and morphology (Beck et al., 2019, 2022; de la Cámara et al., 2023; Leblanc et al., 2011; Skúlason et al., 1989). Interestingly, another member of the glypican family, *gpc4*, appears to have an impact in the craniofacial structures of zebrafish, mostly in the area of the jaw (LeClair et al., 2009), and *gpc3* was identified as a transcriptional regulator associated with an e-QTL hotspot in marine and freshwater stickleback ecomorphs from the Baltic Sea (Pritchard et al., 2017), which differ in body shape and size.

Two out of the three F_{ST} outliers of *gpc6* were found in the 3'-UTR of the gene. The 3' untranslated regions of genes are known to evolve more rapidly than other coding genetic sequences (Berezikov, 2011). These regions are often rich in miRNA binding sites, small post-transcriptional regulators which are widely involved in gene expression tinkering (Berezikov, 2011; Filipowicz et al., 2008; Mayr, 2019). During evolution the generation of novel miRNAs is more likely than the emergence of novel protein-coding genes (Mayr, 2019), thus it has been hypothesised that miRNAs are important for rapid adaptive divergence or explosive radiations (Franchini et al., 2019; Horta-Lacueva et al., 2023; Kapralova et al., 2014; Xiong et al., 2019). The two SNPs in the 3'UTR formed different haplotypes which are present in the four charr morphs in different proportions. One of these haplotypes (SNP_1 = CC and SNP_2 = GG) is only present in the large morphs, (59% in the LB charr and 21.5% in the PI charr) but was not detected in the small ones. This haplotype may be ancestral, or it may have arisen in the PI morph through introgression from the LB.

Despite the fact that the haplotypes mentioned above were located in a functional part of the *gpc6* gene (namely the 3'-UTR), we exercise caution in interpreting the results in light of recent signatures of selection. However, given that *gpc6* is a developmental gene involved in growth and its function is highly conserved throughout vertebrate evolution, we believe that *gpc6* is a promising starting point for investigating growth and body size in salmonids.

In a plausible evolutionary scenario, a large, piscivorous ancestor likely resembling today's anadromous charr, first colonised Thingvallavatn. The benthic morphs diverged from a PL/PI ancestor early in the population history via resource polymorphism, with the LB and SB charr diverging later from one another. While the LB charr represents a classic case of benthivorous fish, the SB is often considered paedomorphic morph (*i.e.*, retaining juvenile morphological characteristics (Eiríksson et al., 1999). While the benthic morphs enjoyed abundant resources in the lava bed, a large PL/PI charr population experienced intense competition for resources (Guðbrandsson et al., 2019; Skúlason et al., 1989). This competitive pressure of juveniles instigated an ontogenetic dietary shift: the fish would leave their juvenile habitat early and learn to feed on sticklebacks, relying of their ancestral propensity to eat fish. The larger size of these fish-eaters likely allowed them to enter the LB morph mating system. Given the putatively small size of the PI population (*pers. obs.*),

mating with LB charr facilitated the process of finding a partner (Sigurjónsdóttir & Gunnarsson, 1989; Foote & Larkin, 1988). Hybridisation between the PI and the LB charr may occur every generation and likely largely depends on resource availabilities. Incorporating genetic material from LB into the PI's genetic pool may confer them with some ability to maintain larger body sizes. Despite the fact that the existing data cannot explain which mechanisms are playing the most decisive roles, we conclude that the ancestral propensity of large size at maturity in the form of standing genetic variation, further interwoven with introgression, phenotypic plasticity and maternal effects, were likely key in the generation of the large-small axis in the Arctic charr morphs from Thingvallavatn (although the cases of parallel genetic and molecular mechanisms seem to be the exceptions to the rule (**Fig. 6**)).

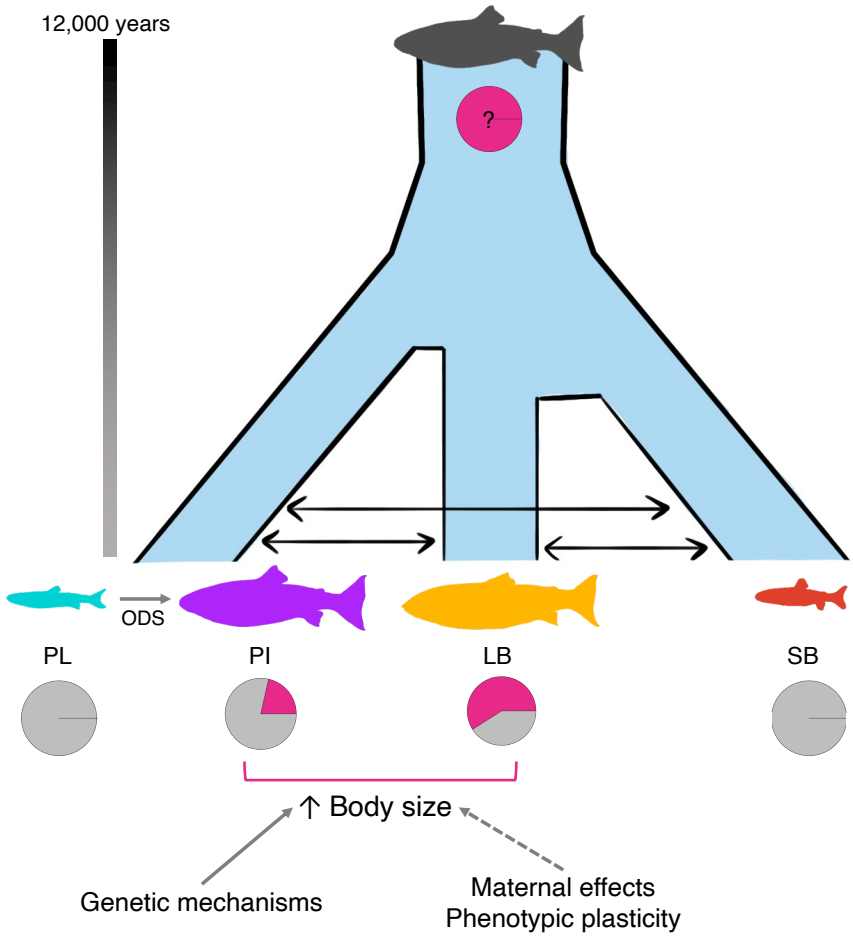

Figure 6. Hypothetical evolutionary framework of the Arctic charr morphs in Thingvallavatn. The anadromous ancestor(s) colonised the lake, which became landlocked soon after the ice receded. The most likely coalescence model (*i.e.,* the limnetic and the benthic populations have an ancestral origin) is depicted in blue. Black arrows represent migration among populations. The pie charts represent the proportion of the GG-CC haplotype (in pink) in the 3'-UTR of *glypican-6* for each morph (unknown in the ancestral anadromous charr). The grey colour represents a mixture of other haplotypes. Genetic mechanisms, phenotypic plasticity and maternal effects play a role in the life history variation of large and small morphs. The dashed arrow indicates processes not directly tested in this study.

Ethical statement

Fish collection was obtained with permissions from the farmers in Mjóanes, and from the Thingvellir National Park Commission. Ethics committee approval is not needed for regular or scientific fishing in Iceland (The Icelandic law on Animal protection, Law 15/1994, last updated with Law 55/2013). All fishing was done with permits from the Directory of Fisheries, SSS held active permits for the sampling period.

Funding

This work was fully funded by the Icelandic Centre of Research, RANNÍS (Icelandic Research Fund grant no. 1535–1533090) to KHK and the University of Iceland‘s Doctoral Fund to MC.

Conflicts of interest

The authors declare no conflict of interests.

Acknowledgements

We would like to thank Arnar Pálsson, Fia Finn, Finnur Ingimarsson, Guðbjörg Ósk Jónsdóttir, Han Xiao, Haraldur R. Ingvason, Lieke Ponsioen, Marcos Lagunas, Nahal Eskafi, Quentin J.B. Horta-Lacueva, Stefán Már Stefánsson and Zophonías O. Jónsson for their sampling efforts in Thingvallavatn. Special thanks to Jóhann Jónsson, farmer at Mjóanes, for his help with the sampling.

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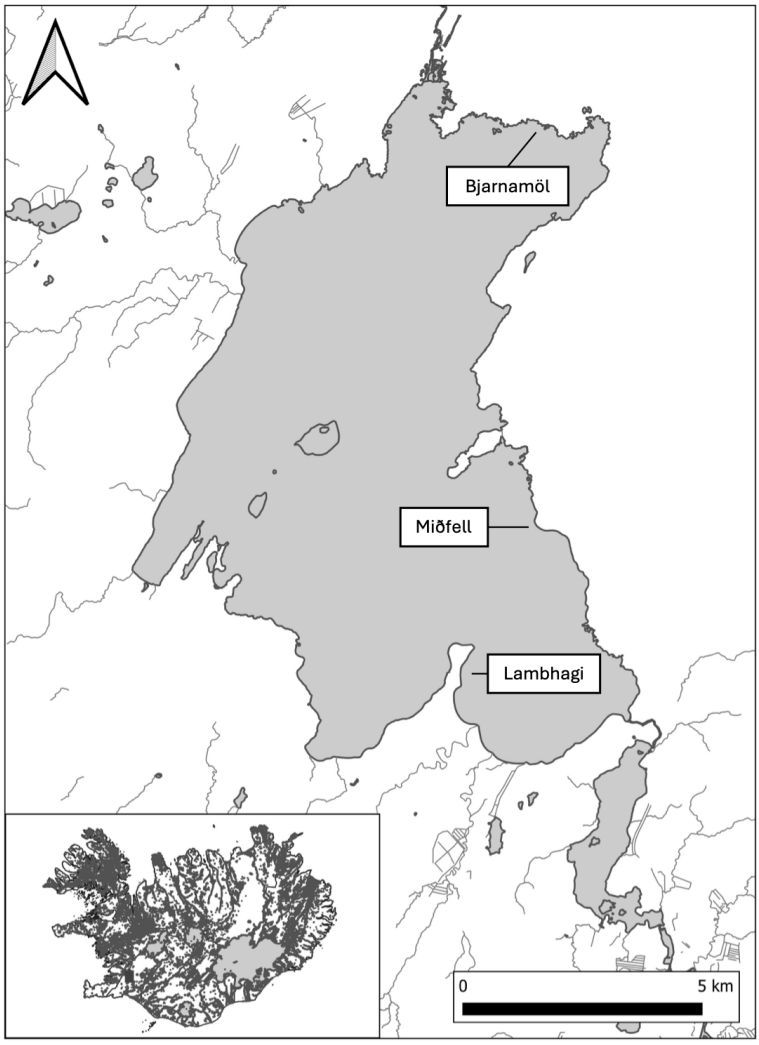
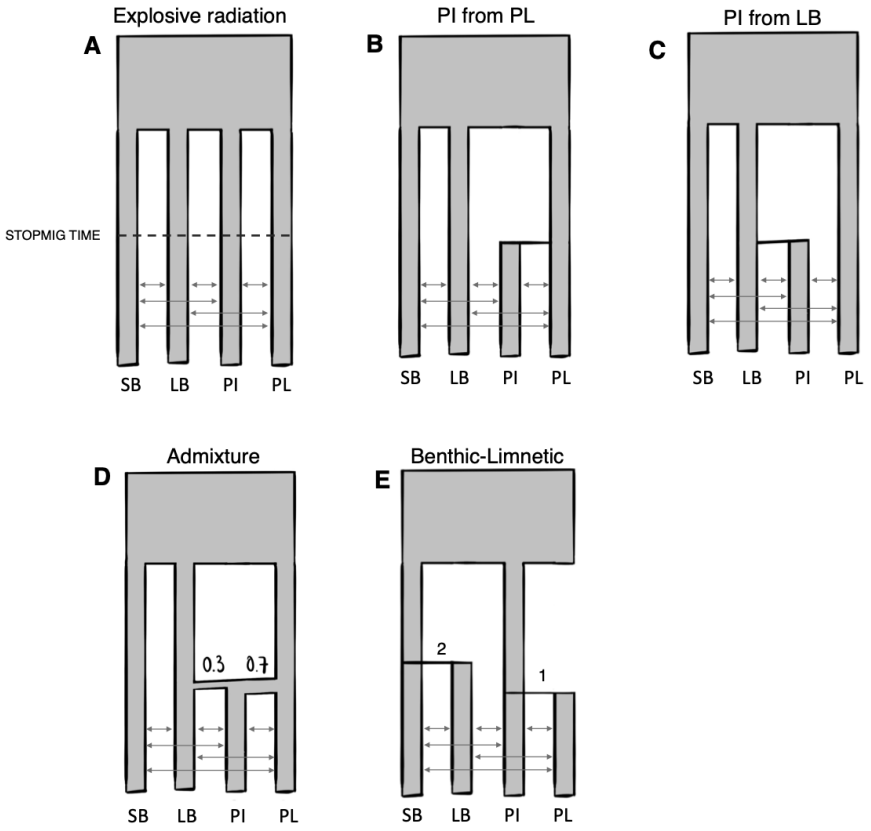
Supplementary material

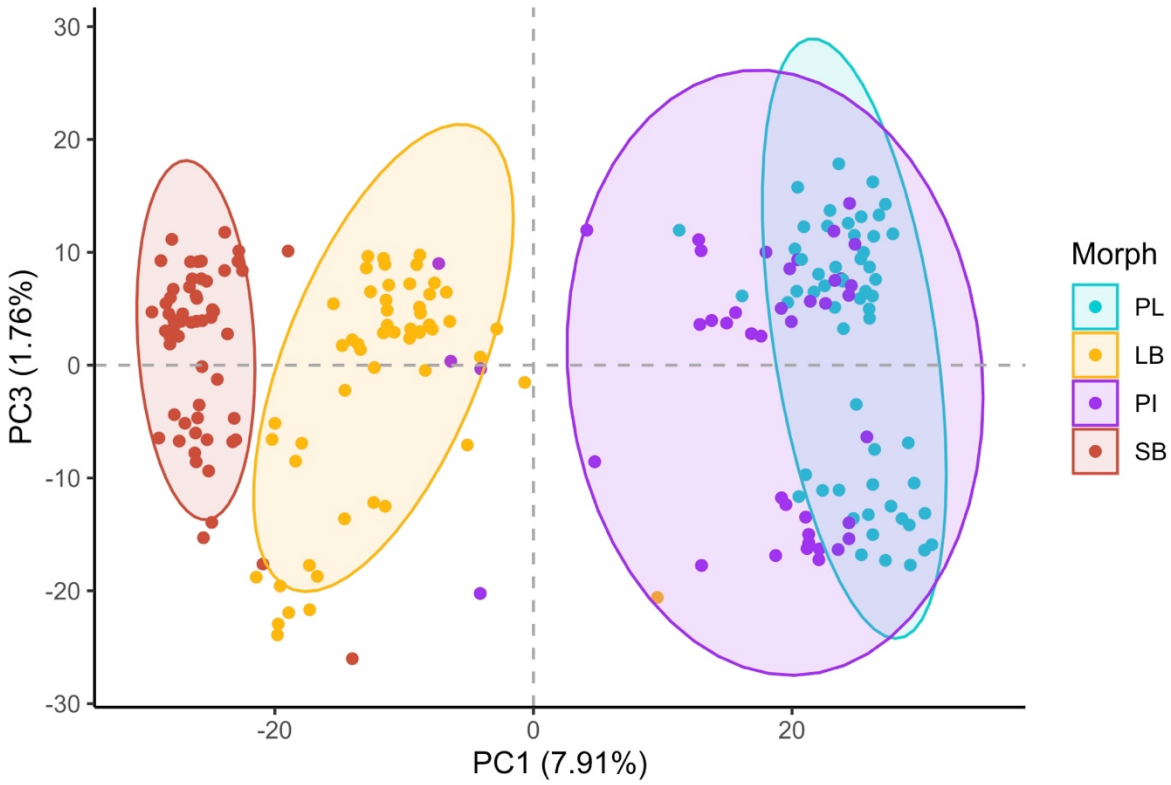


SM Figure 1. Location of the sampling sites in Thingvallavatn. Figure modified from Ponsioen et al., 2020.

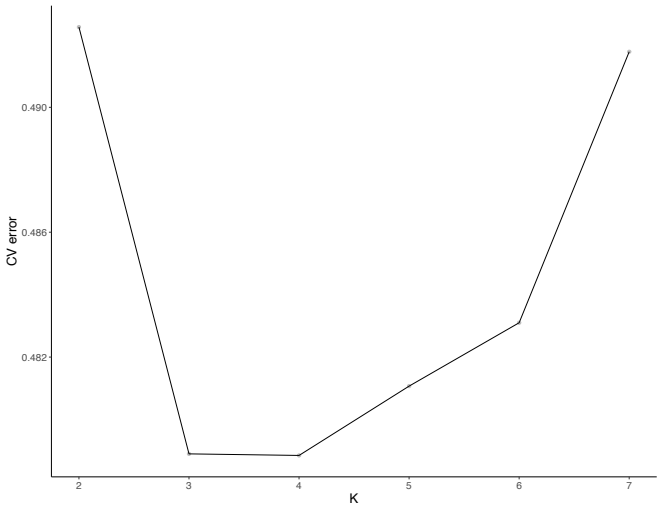
Ponsioen, L. (2020). *Reproductive barriers between sympatric morphs of Arctic charr (Salvelinus alpinus) in lake Thingvallavatn, Iceland* (Masters dissertation).



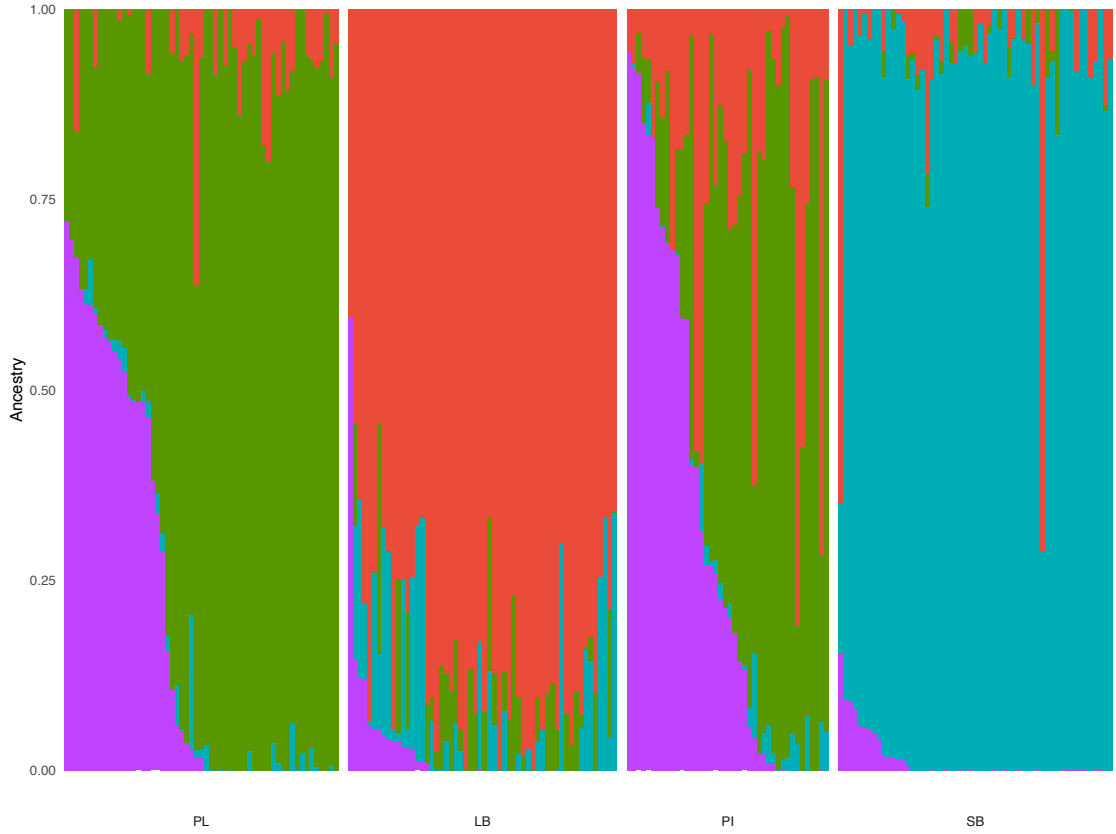
SM Figure 2. The five (A-E) evolutionary scenarios tested in coalescence modelling when PL and PI are set as different genetic populations. We tested an (**A**) explosive radiation model where all four populations diverged at the same time, (**B**) where PI diverges more recently from an ancestral PL/PI population, (**C**) where PI diverges more recently from an ancestral LB/PI population, (**D**) the benthic and the limnetic clusters are ancestral, SB-LB and PL-PI diverging more recenty and (**E**) and admixture model where PI diverges more recently after hybridisation events between PL and LB. All scenarios were tested without and with recent migration with migration rate m(unif,1e-10,0.1) among all pairs (depicted with blue arrows). For model A, migration stops leading to an ancestral allopatry period at an estimated time TSTOPMIG(unif,1,3000), depicted with the red dashed arrow. For models B-D, migration stops after the first divergence event (i.e., PI from PL in B, PI from LB in C and admixture event in D). In E, migration stops after the second divergence event (i.e., 2 (SB-LB) in this figure) but notice that events 1 and 2 do not follow a specific order in time.



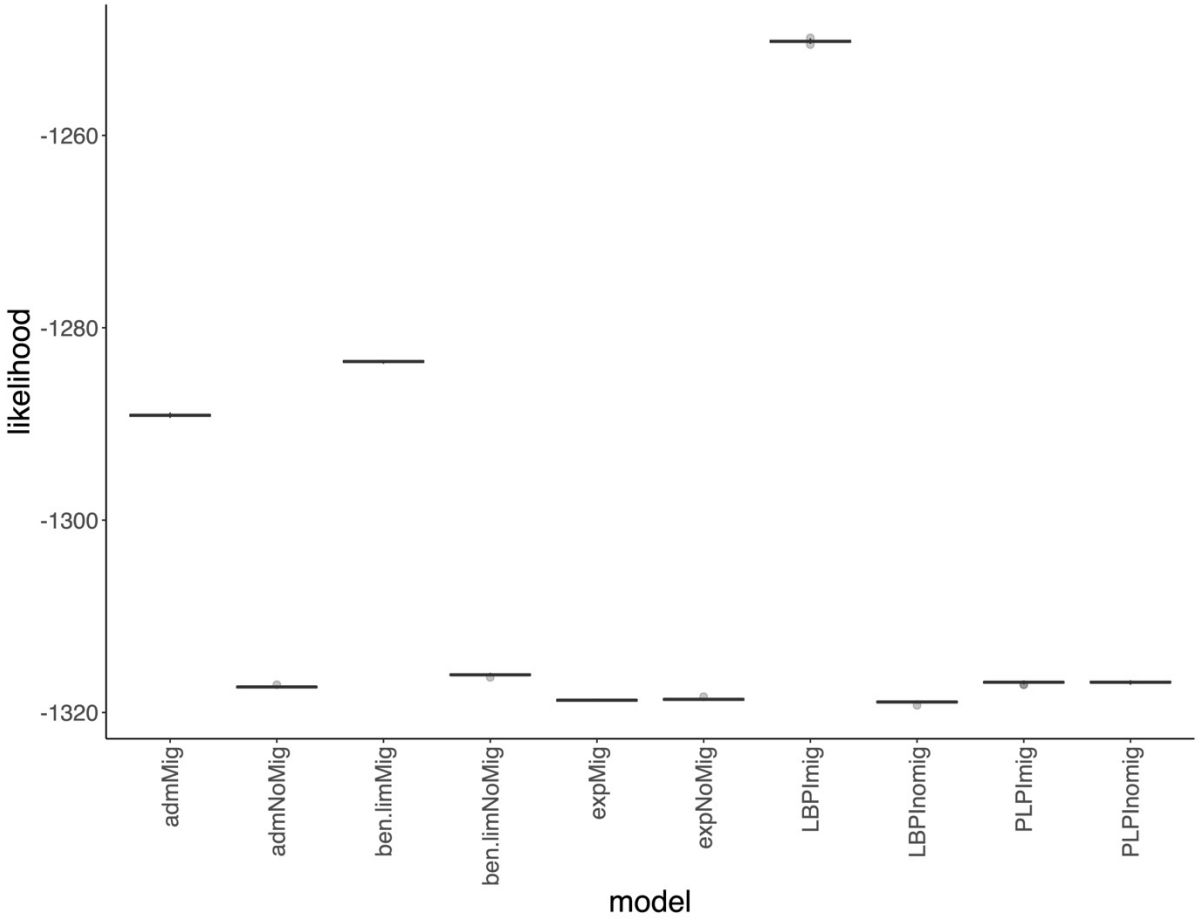
SM Figure 3. Principal Component Analysis (PCA) plots of 212 individuals and 2932 SNPs of principal components 1 and 3. Each point represents one individual, coloured by morph. Shaded, 95% confidence ellipses per morph.



SM Figure 4. Cross-validation errors for each K value (from K=2 to K=7) for ADMIXTURE analysis.



SM Figure 5. ADMIXTURE plot from SNP data for K=4 from 212 genotypes. Each colour represents one cluster and individuals are ordered by their Q values.



SM Figure 6. Boxplot of likelihood distributions of the ten tested coalescence models. The best supported model within each population had the greatest maximum likelihood value. The boxplot whiskers represent 95% confidence intervals for each model.

SM Table 1. Mean F_{ST} values between all population pairs. In parenthesis, confidence intervals calculated on 10,000 permutations between 0.025 and 0.975 quantiles. Asterisks indicate singnificance level (*** = p-value < 0.001, ** = p-value < 0.01 and *ns* = non-significant).

	PL	**SB**	**LB**	**PI**
PL	-			
SB	0.153 (0.145,0.163) ***	-		
LB	0.114 (0.107,0.122) ***	0.075 (0.070,0.081) **	-	
PI	0.011 (0.009,0.012) *ns*	0.122 (0.115,0.130) ***	0.072 (0.067,0.077) ***	-

SM Table 2. Analyses of Molecular Variance (AMOVA) on ≈3000 SNPs using the nested model *size(morph(sample))*.

test	**df**	**Sum Sq**	**Mean Sq**	**Sigma**	**Variation (%)**	**p-value**
Between Size		4878.314	4878.314	-27.617	3.69	0.673
Between Morph Within Size		21122.749	10561.374	93.986	12.55	<0.001
Between samples Within Morph	208	144102.365	692.800	10.282	1.37	0.12
Within Samples	212	142514.054	672.236	672.236	89.765	<0.001
Total	423	312617.481	739.0484	748.888	100.00	

SM Table 3. Post-hoc pairwise-t-test on likelihood~model, without pooling standard deviations and applying bonferroni correction for p adjustment.

Model1	Model2	statistic	df	p	p.adj	p.adj.signif
expMig	expNoMig	2.428	193.76	16	451	ns
expMig	LBancMig	-62.709	145.26	4.35e-107	1.22e-105	****
expMig	LBancNoMig	4.485	197.64	1.24e-5	3.47e-4	***
expMig	PLancMig	-7819.554	197.86	0	0	****
expMig	PLancNoMig	-45.288	195.14	1.76e-105	4.93e-104	****
expMig	SBancMig	2.28	198	24	664	ns
expMig	SBancNoMig	0.74	197.6	0.46	1	ns
expNoMig	LBancMig	-64.066	146.07	8.17e-109	2.29e-107	****
expNoMig	LBancNoMig	2.078	193.99	39	1	ns
expNoMig	PLancMig	-7767.268	193.99	0	0	****
expNoMig	PLancNoMig	-47.28	192.56	6.36e-108	1.78e-106	****
expNoMig	SBancMig	-162	193.78	871	1	ns
expNoMig	SBancNoMig	-1.638	193.98	103	1	ns
LBancMig	LBancNoMig	65.023	148.87	3.37e-111	9.44e-110	****
LBancMig	PLancMig	-4907.773	147.49	0	0	****
LBancMig	PLancNoMig	31.404	155.94	2.56e-69	7.17e-68	****
LBancMig	SBancMig	64.13	145.38	1.64e-108	4.59e-107	****
LBancMig	SBancNoMig	62.6	149.07	6.22e-109	1.74e-107	****
LBancNoMig	PLancMig	-7659.012	197.95	0	0	****
LBancNoMig	PLancNoMig	-48.638	196.78	1.25e-111	3.5e-110	****
LBancNoMig	SBancMig	-2.251	197.66	25	714	ns
LBancNoMig	SBancNoMig	-3.662	198	3.21e-4	9	**
PLancMig	PLancNoMig	7297.995	196.24	0	0	****
PLancMig	SBancMig	7816.608	197.87	0	0	****
PLancMig	SBancNoMig	7646.294	197.93	0	0	****
PLancNoMig	SBancMig	47.399	195.2	4.82e-109	1.35e-107	****
PLancNoMig	SBancNoMig	45.071	196.85	1.13e-105	3.16e-104	****
SBancMig	SBancNoMig	-1.489	197.62	138	1	ns

SM Table 4. ΔAIC and AIC values for each model simulation, calculated for their best independent run, for the three populations models.

model	ΔAIC	AIC
expNoMig	66.180	1084.0421
expMig	66.211	1088.1849
LBancNoMig	66.176	1088.0238
LBancMig	61.077	1066.5420
SBancNoMig	66.178	1088.0330
SBancMig	65.374	1086.3304
PLancNoMig	66.037	1087.3836
PLancMig	40.780	973.0709

SM Table 5. AIC and ΔAIC values for each model simulation, calculated for their best independent run, for the 4 populations models.

Model	ΔAIC	AIC
Explosive radiation (no mig)	369.231	6084.7380
Explosive radiation (mig)	369.244	6090.7979
PI from PL (no mig)	367.310	6079.8914
PI from PL (mig)	367.317	6081.9237
PI from LB (no mig)	369.346	6089.2676
PI from LB (mig)	300.588	5774.6253
Admixture (no mig)	368.005	6085.0920
Admixture (mig)	339.608	5956.3190
Benthic-limnetic (no mig)	366.638	6080.7968
Benthic-limnetic (mig)	320.812	5871.7602

SM Table 6. F_{ST} outliers above a threshold > 97.5%, between PL and PI.

Locus ID	LG	BP	Annotation
84995	LG01	56471181	myosin-binding protein H-like
112751	LG02	16967100	glypican-6
112751	LG02	16967266	glypican-6
112772	LG02	16974455	glypican-6
112772	LG02	16974462	glypican-6
139058	LG02	34389072	rho GTPase-activating protein 20-like
156375	LG03	4888990	tyrosine 3-monooxygenase-like
156430	LG03	4910524	pseudogene
239584	LG04p	26322915	NC
239600	LG04p	26341406	NC
239600	LG04p	26341409	NC
239600	LG04p	26341433	NC
239600	LG04p	26341465	NC
239600	LG04p	26341687	NC
239301	LG04p	26008286	28S ribosomal protein S22
341094	LG04q.1:29	63644270	transmembrane protein 189
285425	LG04q.1:29	26590292	anaphase-promoting complex subunit CDC26
409161	LG04q.2	16624439	NC
409161	LG04q.2	16624574	NC
451112	LG05	17396741	ephrin-A5
451112	LG05	17396793	ephrin-A5
521330	LG06.1	29120286	NC
521334	LG06.1	29120664	NC
503825	LG06.1	17816211	teashirt homolog 1
526172	LG06.2	2104710	uncharacterized LOC111965664
592082	LG07	21129872	signal peptide, CUB and EGF-like domain-containing protein 3
684438	LG08	50826433	KH domain-containing, RNA-binding, signal transduction-associated protein 2
684438	LG08	50826472	KH domain-containing, RNA-binding, signal transduction-associated protein 2
738429	LG09	29964956	NC

738429	LG09	29965108	NC
738517	LG09	30029603	NC
843027	LG11	43844357	disks large-associated protein 4-like
856705	LG12	4372131	inactive dipeptidyl peptidase 10-like
975250	LG14	20451856	failed axon connections homolog
1119398	LG15	64598198	transmembrane protein 41B
1119398	LG15	64598200	transmembrane protein 41B
1119398	LG15	64598437	transmembrane protein 41B
1068819	LG15	30856910	neuropeptide FF receptor 1-like
1068993	LG15	30944346	hexokinase-2
1031356	LG15	8689366	NC
1068709	LG15	30794795	NC
1076333	LG15	35814557	NC
1115089	LG15	61475815	fibulin-7-like
1138727	LG16	9650388	NC
1155660	LG16	19344892	NC
1168961	LG16	27538466	NC
1193729	LG17	6283120	NC
1588372	LG22	33481937	proton-coupled amino acid transporter 4
1626559	LG23	22350995	nuclear receptor corepressor 1
1615856	LG23	15822649	rho GTPase-activating protein 32-like
1619288	LG23	18026148	inactive peptidyl-prolyl cis-trans isomerase FKBP6
1664648	LG23	45761078	NC
1622066	LG23	19642581	pseudogene
1628548	LG23	23565004	pseudogene
1788822	LG26	43605064	eukaryotic translation initiation factor 4 gamma 2
1733298	LG26	8286358	NC
1733298	LG26	8286574	NC
1789311	LG26	43925144	NC
1872821	LG28	11924083	gap junction Cx32.2 protein-like
1877654	LG28	14693537	flvcr1: feline leukemia virus subgroup C cellular receptor 1
1873462	LG28	12337161	protein FAM184A-like,
1915116	LG30	7632164	papd7: poly(A) RNA polymerase D7, non-canonical
1915067	LG30	7597503	NC
1975446	LG31	23405756	NC
1982764	LG31	30165585	NC
2028816	LG32	29092064	NC
2097898	LG33	36050208	scavenger receptor class F member 2-like, transcript variant X1
2092690	LG33	31580495	NC
2099330	LG33	37395343	NC
2092758	LG33	31624209	pseudogene
2092758	LG33	31624232	pseudogene
2092758	LG33	31624236	pseudogene
2092758	LG33	31624237	pseudogene
2126408	LG35	11433557	NC
2178907	LG36	25470503	NC

SM – Table 7. F_{ST} outliers above a threshold > 97.5%, between LB and SB.

Locus ID	LG	BP	Annotation
62193	LG01	42454040	NC
62536	LG01	42649244	NC
69787	LG01	46919971	NC
70638	LG01	47373587	cadherin-4-like
112751	LG02	16967100	glypican-6
112751	LG02	16967266	glypican-6
112772	LG02	16974462	glypican-6
130277	LG02	28346534	ubiquitin-conjugating enzyme E2 E3
131163	LG02	28889889	nck-associated protein 1
131163	LG02	28890005	nck-associated protein 1
131163	LG02	28890012	nck-associated protein 1
131173	LG02	28897181	NC
132813	LG02	29916246	NC
133069	LG02	30050621	NC
133703	LG02	30451874	NC
156430	LG03	4910524	pseudogene
186340	LG03	26555958	NC
321282	LG04q.1:29	51700341	NC
372217	LG04q.1:29	83692422	NC
372217	LG04q.1:29	83692425	NC
411834	LG04q.2	18435494	ral GTPase-activating protein subunit alpha-1
500987	LG06.1	15793166	uncharacterized LOC111965203
500987	LG06.1	15793225	uncharacterized LOC111965203
500987	LG06.1	15793228	uncharacterized LOC111965203
500987	LG06.1	15793253	uncharacterized LOC111965203
542226	LG06.2	13169973	follistatin like 5
542226	LG06.2	13170004	follistatin like 5
545253	LG06.2	15097632	NC
611435	LG08	1436763	NC
612117	LG08	2424463	pseudogene
612252	LG08	2499387	eukaryotic translation initiation factor 4E-binding protein 3-like
612961	LG08	3199795	glypican-4
612963	LG08	3200116	glypican-4
629439	LG08	15338613	NC
686774	LG08	52378348	NC
716142	LG09	15833961	NC
716142	LG09	15834138	NC
716499	LG09	15989748	kinesin-like protein KIF15-B

772748	LG10	20744346	switch-associated protein 70
872727	LG13	2903397	protein FAM19A5
872727	LG13	2903607	protein FAM19A5
1058489	LG15	24759377	AP-1 complex subunit beta-1
1119973	LG15	64984028	NC
1119973	LG15	64984160	NC
1119983	LG15	64987085	NC
1152952	LG16	17690856	NC
1155660	LG16	19344892	NC
1197070	LG17	8433402	NC
1215882	LG17	20402958	uncharacterized LOC111976432
1218595	LG17	21961973	pseudogene
1268018	LG18	14034716	calpain-15
1268018	LG18	14034911	calpain-15
1332327	LG18	56755176	NC
1332327	LG18	56755345	NC
1333630	LG18	57723367	alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase 2
1403797	LG19	34928152	low-density lipoprotein receptor-related protein 8
1630283	LG23	24634535	PR domain zinc finger protein 15-like
1639948	LG23	30716289	connector enhancer of kinase suppressor of ras 2-like,
1656317	LG23	40477970	GDNF family receptor alpha-4
1832683	LG27	22818115	cadherin-18
1832699	LG27	22828047	cadherin-18
1832699	LG27	22828221	cadherin-18
1922297	LG30	12908108	NC
1927487	LG30	16083733	NC
1928218	LG30	16463449	protein AF1q-like
1929245	LG30	17101078	uncharacterized LOC111955387
1929245	LG30	17101224	uncharacterized LOC111955387
1929282	LG30	17124589	uncharacterized LOC111955387
1939164	LG30	23796538	NC
1994497	LG32	7095598	low-density lipoprotein receptor-related protein 8
2094885	LG33	33434754	growth hormone receptor-like
2094893	LG33	33436033	growth hormone receptor-like
2113677	LG35	2487422	amyloid-like protein 2
2165229	LG36	16174700	NC
2174264	LG36	22435337	transmembrane protein FAM155A

SM – Table 8. F_{ST} outlier shared between groups of 2 or 3 large-small pairs of Arctic charr morphs, sorted by linkage groups and number SNPs per region. Coding regions hit by one or more SNP outliers are listed in "In coding region column". The vicinity of the outliers was additionally inspected (±10,000bp away from the SNP).

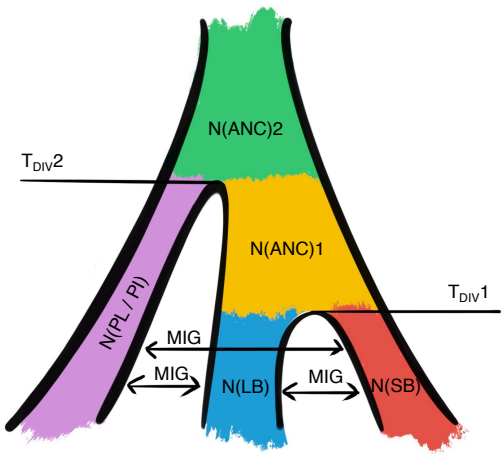
	LG	# SNPs	In region	In vicinity of the region (±10,000bp)
Three pairs				
LB-SB | PL-LB | PL-PI	LG16	1	-	-
PL-LB | PL-PI | SB-PI	LG26	1	eukaryotic translation initiation factor 4 gamma 2	
	LG26	1	-	-
Two pairs				
LB-SB | PL-PI	LG02	3	glypican-6	glypican-6
	LG03	1	pseudogene (exon)	before: nucleoporin 37, transcript variant X1 after: pro-MCH 1-like
LB-SB | PL-LB	LG01	1	-	before: netrin4-like after: mitochondrial intermembrane space import and assembly protein 40
	LG01	1	cadherin-4-like	cadherin-4-like
	LG27	3	cadherin-18	cadherin-18
PL-LB | SB-PI	LG02	1	-	-
	LG04q.1:29	1	NT-3 growth factor receptor-like	NT-3 growth factor receptor-like
	LG04q.2	1	tyrosine-protein kinase receptor TYRO3	tyrosine-protein kinase receptor TYRO3
	LG12	1	uncharacterized LOC111970864	before: cordon-bleu protein-like 1 after: pseudogene
	LG18	1	CUB and sushi domain-containing protein 1-like	CUB and sushi domain-containing protein 1-like
	LG18	1	-	before: serglycin after: VPS26, retromer complex component A
	LG20	1	tyrosine-protein kinase Fer	tyrosine-protein kinase Fer
	LG27	2	-	-
	LG28	4	-	-
	LG30	1	-	-
	LG34	1	15-hydroxyprostaglandin dehydrogenase	15-hydroxyprostaglandin dehydrogenase
	LG36	1	-	-
PL-LB | PL-PI	LG02	1	glypican-6	before: glypican-6
	LG06.1	1	teashirt homolog 1	teashirt homolog 1
	LG06.2	1	uncharacterized LOC111965664	uncharacterized LOC111965664
	LG12	1	inactive dipeptidyl peptidase 10-like	inactive dipeptidyl peptidase 10-like
	LG15	3	transmembrane protein 41B	before: transmembrane protein 41B after: importin-7

	LG16	1	-	-
	LG22	1	proton-coupled amino acid transporter 4	after: proton-coupled amino acid transporter 4
	LG26	2	-	before: syntaxin-6 after: uncharacterized protein KIAA1614-like
	LG28	1	gap junction Cx32.2 protein-like	before1: gap junction alpha-1 protein-like before2: gap junction Cx32.2 protein-like after1: gap junction Cx32.2 protein after3: pseudogene
	LG30	1	-	-
	LG30	1	poly(A) RNA polymerase D7, non-canonical	before: poly(A) RNA polymerase D7, non-canonical after: uncharacterized LOC111954975
LB-SB | SB-PI	LG01	1	-	-
	LG02	3	nck-associated protein 1	nck-associated protein 1
	LG02	1	-	before: nck-associated protein 1 after: secreted frizzled-related protein 3-like
	LG02	1	-	after: fibrous sheath CABYR-binding protein
	LG02	1	SH3 domain-containing RING finger protein 3-like	SH3 domain-containing RING finger protein 3-like
	LG02	1	-	-
	LG04q.2	1	ral GTPase-activating protein subunit alpha-1	ral GTPase-activating protein subunit alpha-1
	LG08	1	eukaryotic translation initiation factor 4E-binding protein 3-like	before: pseudogene after: eukaryotic translation initiation factor 4E-binding protein 3-like
	LG09	2	-	before: protein phosphatase 1A
	LG09	1	kinesin-like protein KIF15-B	before1: ATP-dependent RNA helicase TDRD9-like before2: protein RD3-like after: kinesin-like protein KIF15-B
	LG10	1	switch-associated protein 70	before: switch-associated protein 70
	LG15	1	AP-1 complex subunit beta-1	AP-1 complex subunit beta-1
	LG15	3	-	-
	LG17	1	uncharacterized LOC111976432	before: forkhead box protein P1-B after: uncharacterized LOC111976432
	LG18	2	calpain-15-like	calpain-15-like
	LG23	1	PR domain zinc finger protein 15-like	after: uncharacterized LOC111951086
	LG23	1	connector enhancer of kinase suppressor of ras 2-like	after: MICOS complex subunit MIC27
	LG30	1	-	after: protein disulfide-isomerase A3

	LG30	1	protein AF1q-like, transcript variant X1	before: CDC42 small effector protein 1 after: GA-binding protein subunit beta-1
	LG30	1	-	-
	LG36	1	transmembrane protein FAM155A	transmembrane protein FAM155A
PL-PI | SB-PI	-	1	-	-

SM 9. Mean parameter estimates and their upper and lower 95% confidence intervals from backward simulation on the most likely model (LBPImig). To account for linkage among SNPs, 50 random bootstrap blocks were generated from the same genomic data. The model PLancMig was re-run 100 times for each bootrstrap block and the best run was selected. We then bootstrapped (10,000 repetitions) each mean for all the parameters to obtain confidence intervals.

Parameter	Mean	Lower CI	Upper CI
N(PL/PI)	61184	43365	84092
N(LB)	3592712	330579	4365173
N(SB)	116.3	105	239
N(ANC)1	793	70	23153
N(ANC)2	74439980	58968977	75995712
TDIV1	2171	1528	2815
TDIV2	4708	3012	5001
MIG	4.150e-05	6.967e-06	5.676e-5



N(POP) = effective population size at present. This parameter search range (for all three populations) is a log-uniform distribution with a minimum of 100 individuals and a maximum of 10 million.

N(ANC)(1,2) = effective population size of the ancestral populations 1 and 2. The search range is a log-uniform disitribution with a minimum of 10 individuals and a maximum of 10 million.

TDIV1 = time of divergence (in generations) of N(ANC)1 into LB and SB. The search range for this parameter is a uniform distribution of a minimum of 1 generation and a maximum of 3000.

TDIV2 = time of divergence (in generations) of N(ANC)2 into PL/PI and N(ANC)1. TDIV2 responds to this formula: TDIV2 = TDIV1 + TPLUSDIV where TPLUSDIV has a search range of a uniform distribution of a minimum of 1 and a maximum of 3000 generations.

MIG = migration rate among morphs. The search range is a log-uniform distribution of a minimum of 1e-10 and a maximum of 0.1 migration rate.