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Evolutionary ecogenomics of North Atlantic ptarmigan

Theodore E. Squires

DOCTORAL THESIS

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Evolutionary ecogenomics of North Atlantic ptarmigan

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Natural Resource Sciences

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Þróunarfræðileg visterfðamengjafræði rjúpu í kringum Norður-Atlantshaf

Theodore E. Squires

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The Virgin Mary and the Ptarmigan.

— Once, the Virgin Mary summoned all the birds to meet before her. When they came thither, she commanded them to wade through flaming fire. Knowing her to be queen of heaven, and a powerful queen too, they dared not to disobey her order, and jumped all into the fire and through it, save the ptarmigan. When the other birds came through the fire, their feet were scorched to the skin, and have remained so ever since. But the ptarmigan, the only disobedient bird, did not fare a whit better, albeit she got no scorched feet ; for Mary grew angry with her, and laid upon her the curse, that she should be the greatest of faint-hearts, the most harmless and defenceless among birds, on the one side, but, on the other, the most persecuted, and should never enjoy an hour in her life free from the fear of being persecuted, save on the day of Whitsuntide. The falcon should be her worst foe, and most dangerous persecutor, and constantly prey upon her flesh. But, so far had Mary mercy upon the ptarmigan, that the bird should be allowed to change colour, according to the seasons, being white as the snow in winter, but brown-gray as the heather in summer. This curse and mercy have ever since rested upon the poor ptarmigan, by the power of the unchangeable act of Mary the queen of heaven. The falcon, which before this sentence was passed was the brother of the ptarmigan, never remembers his kinship to his sister, till he comes to her heart. For then breaks his sorrow first forth, as it comes into his mind that he has eaten his sister.

From Icelandic Legends (Collected by Jón Árnason)
Translation by George E. J Powell and Eiríkur Magnússon - 1866.

Acknowledgments

Deepest thanks to my colleagues and the administrative faculty at the University of Akureyri and Uppsala University for showing a sincere interest in my academic progress, true friendship, and for working together to provide an exceptional opportunity for me to develop this thesis. Allowing me to pursue a joint degree in the present fashion undoubtedly benefitted the quality of my work and education.

My main supervisors Kristinn Pétur Magnússon and Jacob Höglund were in lock-step throughout the program and together they‘ve been unmatchable professional role models. This whole project would probably have been nothing more than a “pile of sundry facts” without the skills, supervision, and mentorship of Patrik Rödin-Mörch. My other co-supervisors Snæbjörn Pálsson, Eva Halapi, and Jennifer Forbey proved to be helpful sounding boards and offered excellent suggestions throughout the program. Additional gratitude goes to Zophonías Oddur Jónsson who helped me find direction along the way.

This work was ultimately funded by Icelandic and Swedish tax payers through the Icelandic Research Fund (Rannís) and Swedish Research Council (Vetenskapsrådet). I received additional support from the University of Akureyri Travel Fund, the Uppsala Student Union Nordic Travel Scholarship, and multiple consecutive scholarships from the Zoological Research Foundation (Stifelsen Zoologisk forskning).

Closer to home I‘ve been consistently supported by my family to pursue studies while my kind and patient partner Camille has stood beside me and brought me snackies through every looming deadline. Ilsa Birmingham was a particularly influential science educator among many others in my life, her own joys sparked mine.

Thirteen years ago I stumbled into the laboratory of Ólafur Karl Nielsen at the Natural Science Institute of Iceland while on a short visit to this wonderful country. I never could have guessed how a few weeks in a ptarmigan lab might snowball so many years later. His hospitality ultimately brought me back to Europe and into this doctoral research position. I hope to pay it forward.

Abstract

This thesis integrates a new chromosome level reference genome with population and temporal whole genome resequencing data to understand adaptation, demographic dynamics, and conservation risks for Rock Ptarmigan (*Lagopus muta*) in Iceland and around the North Atlantic. Paper I delivers a high quality 1.03 Gb assembly and annotation that enables robust mapping and functional interpretation. Using 99 ptarmigan genomes from nine countries, two climate products (CHELSA, WorldClim), and two statistical tools (BayPass, LEA) Paper II shows that climate associated variation is broadly polygenic and that genomic offset forecasts are highly sensitive to climate dataset and association method, yielding divergent vulnerability maps for Arctic and alpine populations. Paper III leverages 91 juvenile birds from an 11-year time series to demonstrate pervasive, rapid allele frequency oscillations aligned with multi annual population cycles; many fluctuating loci map to neurological, behavioral, and immune pathways, consistent with density dependent selection maintaining polymorphism. Paper IV reports an exploratory GWAS on 90 juveniles, finding no single large effect loci for condition traits (fat, size, weight) and supporting a highly polygenic architecture; however, plausible candidate genes (e.g., *HIVEP3*, *HTR1F*, *LAMA3*, *GALNT9*, *ACSL3*, *EBF1*) offer hypotheses for targeted follow up. Across papers the work yields practical guidance for future conservation work: prioritize ensemble climate models, expand temporal genomic monitoring, preserve functional genetic diversity, and develop regionally informed management. Together, these studies provide genomic resources, analytical workflows, and an empirically grounded framework for forecasting and managing ptarmigan populations under rapid environmental change.

Ágrip

Í þessari doktorsritgerð er nýtt viðmiðunarerfðamengi notað til að samþætta heilerfðamengjagögn úr stofnum og tímaseríum til að skilja aðlögun, lýðfræðidýnamík, og þá vá sem steðjar að framtíð fjallrjúpunnar (*Lagopus muta*) á Íslandi og í stofnum kringum Norður Atlantshaf. Í grein I er lýst hágæða 1,03 Gb erfðamengi rjúpunnar með kortlögðum genum á litninga sem gerir virknirannsóknir á erfðaefninu mögulegar. Í grein II eru borin saman erfðamengi 99 rjúpna frá níu löndum við Atlantshaf, með því að nota tvö loftslagsgagnasett (CHELSA, WorldClim) og tvö tölfræðiforrit (BayPass, LEA). Niðurstöður sýna með útreikningum að stofnarnir eru mjög aðlagaðir loftslagsskilyrðum, á fjölgenagrunni, og nota má erfðabreytileikamat til að spá fyrir um aðlögunarhæfni í hlýnandi loftslagi (genomic offset), sem er háð vali á loftlagsgögnum og fylgniaðferðum. Stofnar á heimskauta- og fjallasvæðum virðast sérstaklega viðkvæmir fyrir hlýnun. Í grein III eru rannsakaðar samsætutíðnibreytingar 91 erfðamengis í stofnsveiflum yfir ellefu ára tímabil. Niðurstöðurnar sýna sveiflur í takt við hæðir og lægðir stofnsins, og fylgni við erfðabreytileika í genum sem tengjast tauga-, atferlis- og ónæmisfræðilegum hlutverkum, sem eru í samræmi við þéttleikaháð val sem viðheldur erfðabreytileika. Grein IV fjallar um fylgnigreiningu með erfðamengjaskimun (GWAS) á 90 ungfuglum við ástandsbreytur (fitu, stærð, þyngd), þar sem sterk áhrif einstakra lókusa koma ekki fram, sem styður fjölgenavirkni, til að nefna áhugaverð kandídatsgen (t.d. HIVEP3, HTR1F, LAMA3, GALNT9, ACSL3, EBF1) sem bjóða upp á tilgátur fyrir markvissa eftirfylgni í stærra úrtaki. Doktorsritgerðin er hagnýtur vegvísir fyrir framtíðarnáttúrvernd, hvernig megi forgangsraða erfðamengjagrunduðum loftslagslíkönum, nota erfðamengjafræði í vöktun stofna yfir tíma, varðveita virkni erfðabreytileika og þróa svæðisbundna stjórnun til verndunar. Rannsóknirnar hafa leitt til erfðamengjasafna, greiningarferla og ígrundaðs ramma til að spá fyrir um breytingar á rjúpnastofnum og hvernig megi viðhalda þeim í örum umhverfisbreytingum.

Sammanfattning

Denna avhandling integrerar ett nytt kromosomnivå-referensgenom med populations- och tidsseriebaserade helgenomssekvenseringsdata för att förstå anpassning, demografiskdynamik och bevaranderisker för fjällripa (*Lagopus muta*) på Island och runt Nordatlanten. Artikel I presenterar en högkvalitativ 1,03 Gb-assembly och annotering som möjliggör robust kartläggning och funktionell tolkning av DNA-data. Med 99 ripegenom från nio länder, två klimatdatabaser (CHELSA, WorldClim) och två statistiska verktyg (BayPass, LEA) visar artikel II att klimatassocierad variation i hög grad är polygen och att prognoser för genomisk offset är mycket känsliga för val av klimatdatamängd och associationsmetod, vilket ger upphov till divergerande sårbarhetskartor för arktiska och alpina populationer. Artikel III utnyttjar sekvenseringsdata från 91 juvenila fåglar från en 11-årig tidsserie för att visa genomgripande, snabba oscillationer i allelfrekvenser som följer fleråriga populationcykler; många fluktuerande loci kartläggs till neurologiska, beteendemässiga och immunsystemrelaterade funktioner, vilket är förenligt med att täthetsberoende selektion upprätthåller polymorfism. Artikel IV rapporterar en explorativ GWAS på 90 juveniler och finner inga enskilda loci med stor effekt för konditionsegeneskaper (fett, storlek, vikt), vilket stöder en starkt polygen arkitektur; dock ger plausibla kandidatgener (t.ex. *HIVEP3*, *HTR1F*, *LAMA3*, *GALNT9*, *ACSL3*, *EBF1*) anledning till riktade uppföljningar. Översiktligt ger avhandlingen praktisk vägledning till framtida naturvårdsarbete: prioritera ensemblebaserade klimatmodeller, utöka tidsseriebaserad genomisk övervakning, bevara funktionell genetisk mångfald och utveckla regionalt informerad förvaltning. Tillsammans tillhandahåller dessa studier genomiska resurser, analytiska arbetsflöden och ett empiriskt förankrat ramverk för att prognostisera och förvalta ripbestånd under snabb miljöförändring.

Contents

List of abbreviations

SNP – Single Nucleotide Polymorphism
GWAS – Genome Wide Association Study
HTR1F – 5-hydroxytryptamine (serotonin) receptor 1F
HIVEP3 – HIV Enhancer-Binding Protein Zinc Finger 3
LAMA3 – Laminin Subunit Alpha 3
GALNT9 – Polypeptide N-acetylgalactosaminyltransferase 9
ACSL3 – acyl-CoA synthetase long chain family member 3
EBF1 – Early B-Cell Factor 1
CTNNA2 – Catenin Alpha 2

List of published sequences

The reference genome assembly, including the raw shotgun sequencing data, has been uploaded to NCBI and is avail able at https://www.ncbi.nlm.nih.gov/assembly/GCA_023343835. 1; BioProject: PRJNA836583; BioSample: SAMN25144835. The mitochondrial assembly is publicly available in NCBI and is avail able at https://www.ncbi.nlm.nih.gov/nuccore/OQ580988.1.

Other Repository Details

All scripts associated with the current dissertation and published papers are maintained on a public GitHub repository available here: https://github.com/RockPtarmigan

List of tables and figures

Overview of original articles

This thesis is based on four included papers. Hereafter they will be referred to by their numbers as follows:

Paper I: Theodore E Squires, Patrik Rödin-Mörch, Giulio Formenti, Alan Tracey, Linelle Abueg, Nadolina Brajuka, Erich Jarvis, Eva C Halapi, Páll Melsted, Jacob Höglund, Kristinn Pétur Magnússon. 2023. A chromosome-level genome assembly for the Rock Ptarmigan (*Lagopus muta*), G3 Genes|Genomes|Genetics, Volume 13, Issue 7: jkad099, https://doi.org/10.1093/g3journal/jkad099

Paper II: Theodore E Squires, Patrik Rödin-Mörch, Kristinn Pétur Magnússon, Jacob Höglund. 2025. Genomic vulnerability in Arctic birds is driven by polygenic adaptation and confounded by climate model choice. Evolutionary Applications (In Review).

Paper III: Theodore E Squires, Wanyi Wei, Alexandre A Rêgo, Patrik Rödin-Mörch, Jacob Höglund, Kristinn Pétur Magnússon. 2026. Understanding allele frequency shifts under demographic cycling in Icelandic rock ptarmigan. Heredity (In Review). https://doi.org/10.21203/rs.3.rs-8090944/v1

Paper IV: Theodore E Squires, Athanasios Toros, Ólafur Karl Nielsen, Guðmundur Guðmundsson, Patrik Rödin-Mörch, Jacob Höglund, Kristinn Pétur Magnússon. 2026. Exploring phenotypic trends and genotypic relationships through genome wide investigation of Icelandic ptarmigan. (Manuscript).

Declaration of contribution to the thesis

The following authors have contributed to the main papers and manuscripts for this thesis:

Linelle Abueg (LA), Nadolina Brajuka (NB), Giulio Formenti (GF), Guðmundur Guðmundsson (GG), Eva C Halapi (EH), Jacob Hoglund (JH), Erich Jarvis (EJ), Kristinn Pétur Magnusson (KPM), Páll Melsted (PM), Ólafur Karl Nielsen (ÓKN), Alexandre A Rêgo (AR), Patrik Rodin-Morch (PRM), Theodore Edgar Squires (TES), Athanasios Toros (AtT), Alan Tracey (AlT), and Wanyi Wei (WW).

Paper I: TS and PRM co-first authored this manuscript with TS drafting the main text alongside PRMs interpretive input and summary plot development. TS was responsible for accessioning the mitochondrial data. EJ, GF, NB, were involved with bioinformatic preparation of the reference material along with PM who validated upstream and downstream results. LA and AlT were involved with annotation and pipeline development at the Sanger Institute. EH along with JH and KPM were responsible for funding the reference level sequencing effort with KPM driving the provisioning of the reference samples. EH, JH, KPM, PRM, and TS provided thorough edits for the final version of the manuscript.

Paper II: TS and PRM performed bioinformatics and analysis of population genetics and wrote the first draft of the paper. Specifically, TS prepared this manuscript based on an earlier partially associated preprint. TS was responsible for running of LEA analysis and preparation of climatic datasets while PRM was responsible for mapping, variant calling, and filtering of genetic data in addition to guiding downstream analytical approaches and calculations with BayPass. JH and KPM conceptualized the study, suggested analytical approaches and interpreted the data. All authors were involved in writing the final draft.

Paper III: TS ran analysis and drafted the bulk of this manuscript based on the master's thesis work of WW whose methods were partially retained. AR was responsible for important analytical inquiry into the effects of drift and sampling error and provided methods and additional results based on this. PRM was responsible for bioinformatic preparation of genetic data and variant calling. PRM, JH, and KPM conceptualized the study, suggested analytical approaches and interpreted the data. All authors contributed to the final version of the manuscript.

Paper IV: TS ran a statistical analysis of phenotype data and drafted the manuscript in addition to consulting with AtT on investigative avenues and provisioned working files, AtT was responsible for production and execution of GWAS on the genetic dataset and contributed to writing the methods and provisioning some figures. ÓKN collected phenotypic data and provisioned genetic material along with GG. PRM, JH, and KPM conceived the project and provided oversight across the manuscript's development. AtT, JH, KPM, PRM, TS, and ÓKN contributed to revision and writing of the final version.

1 Introduction

Population genetics today allows for understanding and support of natural systems and provides tools for a deep understanding of life. Considering the present biodiversity crisis and widespread risks that natural systems are facing across the globe as a result of human activities (Cardinale *et al.* 2012), there is a need to recognize the drivers of the present biodiversity loss. Conservation efforts for species, ecosystems, and entire regions should benefit from the insights that ecology and genomics can provide. These benefits may not only come from an immediate understanding of present conditions, but from the eventual prediction of eco-evolutionary trends.

This dissertation primarily deals with the intersection between ecology and genomics and is approached through the lens of a system with great potential. The research pushes towards improving our understanding of the eco-evolutionary dynamics of Rock Ptarmigan (*Lagopus muta*) populations found on Iceland (ssp. *L. m. islandorum*), however broader contributions are made towards eco-evolutionary theories and the findings may have wider applicability.

The field of genomics has exploded in recent decades and with emergent tools this work intends to add new molecular aspects to a field of research that has previously been developed around ptarmigan life history and population dynamics. While the study species represents an interesting system on its own, the contents of this dissertation also contribute valuable insights to evolutionary biology. As Theodosius Dobzhansky wrote in 1973, “Seen in the light of evolution, biology is, perhaps intellectually the most satisfying and inspiring science. Without that light it becomes a pile of sundry facts-- some of them interesting or curious but making no meaningful picture as a whole.” (Dobzhansky 1973). By examining adaptive changes and genetic turnover in ptarmigan, the evolutionary mechanisms responsible for the success and persistence of populations beyond the study system may be elucidated.

I will discuss the goals of this thesis and provide a broad definition of ecogenomics. This is followed by an account of the general biology of the genus *Lagopus*. I next focus on the complex life history of Rock Ptarmigan in Iceland before addressing topics of eco-evolutionary theory including the genetics of demographic cycling and Chitty’s (1957) theory of behavioral polymorphism.

This is followed in the methods section by an overview of the molecular biology tools used to help underpin the scope of this research within evolutionary biology. I then summarize the findings and contributions of each main paper. The final section of the dissertation discusses how this work contributes to the understanding of adaptive evolution and its implications for species conservation.

Thereafter each of the four main manuscripts are presented in turn, along with supplementary tables and figures.

1.1 Major Thesis Objectives

In this dissertation I aim to utilize an ecogenomics framework to explore the evolutionary responses of ptarmigan to environmental changes. New genetic resources and tools are made available through this work. It is made up of several parts in the form of published, submitted, and developing manuscripts as follows:

Paper I: A reference genome from an Icelandic Rock Ptarmigan allowing for an in-depth capacity to map population genomic reads for ecogenomic studies.

Paper II: Regional investigations of local adaptations and genomic offset predictions for various ptarmigan populations around the North Atlantic.

Paper III: Investigation of allele frequency changes in the Icelandic Rock Ptarmigan population in response to cyclical population dynamics.

Paper IV: An exploratory genome-wide association study on phenotypic traits of Icelandic Rock Ptarmigan collected concurrently with population peaks and troughs.

Ultimately, I want this work to achieve several specific goals:

1. Contribute to the understanding of local adaptations across the range of a group of closely related species.
2. Provide evidence of genetic basis for phenotypic responses to shifting environmental pressures and identify possible genes and pathways associated with those responses.
3. Offer new insights that address ongoing questions about the genetics of cycling populations and density dependent selection.
4. Quantify diversity and structure among ptarmigan across the North Atlantic.
5. Understand regional population responses and resilience to anticipated climate change.
6. Enhance understanding of the conservation and genomic health of Icelandic Rock Ptarmigan.

Together, these objectives converge on a broader goal: to provide an integrative framework for conservation genomics that emphasizes both the potential and limitations of current predictive methods. By situating genomic findings within

ecological realities, the thesis contributes to more nuanced, evidence-based strategies for managing biodiversity in rapidly changing environments.

1.2 Ecogenomics

Ecology is often defined as "the relationship among organisms and their environment" (Kloet *et al.* 2011) while genomics can be described as a field of molecular biology concerned with "structure, function, mapping, and evolution of genomes" (Griffiths 2025, Williams *et al.* 2020). Independently the fields of ecology and genomics have experienced rapid maturation in the last century, making both scientific frontiers ripe for exploration. The field of genomics has moved from basic allele quantifications using genetic markers (Hubby & Lewontin 1966) and into the era of next-generation sequencing (Heather & Chain 2016). In ecology there as has been a parallel shift from species descriptions and basic behaviors into holistic integrative models of trophic cycling (Lindeman 1942, Cook 1977) and critical understanding of the anthropogenic impacts on ecosystems (Hansen *et al.* 2013, Cardinale 2012). It is now possible to start understanding the reciprocal influence between ecosystems and genomes.

In this thesis I use the framework of ecogenomics, a unifying idea essentially coined in 2003 that aims to place molecular studies within ecological perspectives (Feder & Mitchell-Olds 2003). Broadly, in ecogenomics, genetic information is used to study the functioning of ecosystems and interactions between species and their environments. In its original iterations there was a focus on how polymorphisms might affect phenotypes in nature (Feder & Mitchell-Olds 2003), though these concepts had been developed for decades prior (Ford 1964). The scope has since expanded away from reductionism to unifying theories that had long been pervasive in molecular biology, and towards understanding the balance of interactions systemically (Van Staalen & Roelofs 2006).

Today, ecogenomics is applied to diverse areas, including the study of nanobacteria in lakes (Chiriac *et al.* 2022), viruses in the ocean (Roux *et al.* 2016), plant-herbivore interactions (Kant & Baldwin 2007), and human health initiatives (Capps *et al.* 2025). The term, while necessary for contextualization, has nebulous edges. I aim to employ this framework to study genetic changes in response to abiotic and biotic variation. By critically analyzing species ecology and the pressures driving natural selection, I can start to theorize about how the "minute orchestra of genes" may play a role in fitness and survival in natural systems with complex life histories.

1.3 Summary of the Genus *Lagopus*

The ptarmigan of the genus *Lagopus* provide an excellent study system to address questions in ecogenomics. The group so named for the fact that all the species of the clade have feathered feet (name derived from Ancient Greek lagos (λαγος; rabbit) + pous (πους; foot); Brisson 1760) is a cold-adapted group with a global distribution above 30 degrees N and is currently broken into four distinct species. These are the Willow Ptarmigan (*L. lagopus*), Rock Ptarmigan (*L. muta*), White-tailed Ptarmigan (*L. leucura*), and Red Grouse (*L. scotica*). The taxonomic species status of the Red Grouse is currently debated (Sangster *et al.* 2022).

Similarities observed across the group include common parasites, demographic population cycling, and many aspects of breeding biology. They also all have 3 cycle molt patterns which is unique among Galliformes. Within the Ptarmigan clade some interspecific interactions are documented, in particular there is evidence of competitive exclusion (Mandeville *et al.* 2024). Some rare interbreeding is known to occur in areas where the species come into contact though species boundaries appear stable although some introgression occur (Quintela *et al.* 2010b). In sympatric populations Rock Ptarmigan often occupy higher elevations at and above the tree line while they are completely outcompeted by Willow Ptarmigan below (Mandeville *et al.* 2024). In areas where Willow Ptarmigan do not occur (such as Iceland, Svalbard, and Greenland), the Rock Ptarmigan are observed from the highest peaks, down to sea level. In most jurisdictions, ptarmigan are classic quarry species and they have been hunted by settlers and indigenous populations for millennia.

According to the IUCN/SSC BirdLife WPA Grouse Specialist Group, ptarmigan populations are nationally red listed in 13 different countries (Storch 2007) though more recently Andorra and Ireland have joined that list (McMahon *et al.* 2012, Government of Andorra 2024; Table 1). Among *Lagopus* species, the Rock Ptarmigan is threatened across the largest area and may face more significant challenges in the face of future climate change due to being the most cold-adapted member of the clade. In general, threats are recognized to include tourism development, hunting, and general habitat degradation (Storch 2007). More specifically, Rock Ptarmigan are recognized to have risks associated with development of skiing infrastructure (Bech *et al.* 2012, Brambilla & Roseo 2025). Among the other species, White-tailed Ptarmigan has received the least attention although the subpopulation in British Columbia is considered regionally threatened (Martin *et al.* 2004) and the Mt. Rainier White-tailed Ptarmigan (*L. leucura rainierensis*) was placed on the Federal Endangered Species List in the United States. (U.S. Fish & Wildlife Service 2024). Despite protections and extremely active support, some populations such as the Rock Ptarmigan in Japan are experiencing continuing declines (Suzuki *et al.* 2013, Fujii *et al.* 2022).

Nationally Red-listed Ptarmigan (*Lagopus spp.*)

Species	Binomial	Nations Red-listing
Willow Ptarmigan	*L. lagopus*	Belarus, China, Estonia, Latvia, Lithuania
Rock Ptarmigan	*L. muta*	Andorra, China, Germany, Iceland, Italy, Japan, Portugal, Slovenia, Spain
White-tailed Ptarmigan	*L. leucura*	*-No National Protections-*
Red Grouse	*L. scotica*	Ireland

Table 1. *Chart providing overview of nationally red-listed ptarmigan populations (Lagopus spp.) according to a variety of sources (Storch 2007, McMahon et al. 2012, Government of Andorra 2024). Several national populations may be re-evaluated in the near future and others remain unassessed.*

Figure 1. *A rare late summer Rock Ptarmigan in Encamp, Andorra (Personal Photo / T. Squires)*

1.3.1 Rock Ptarmigan

The focal species for these studies, the Rock Ptarmigan (*Lagopus muta*) is an alpine and high-arctic specialist often inhabiting exposed unvegetated terrains above the tree line. The species broadly above occur north of 35° N across Eurasia and North America. Notable local populations include those in the Aleutian Islands and Greenland, along with the only terrestrial birds overwintering on Svalbard. The southernmost populations appear to be remnants from the last glacial period now trapped on 'sky islands' in the Pyrenees, Japanese Alps, and European Alps. A detailed distribution map is provided in Paper I. Despite recent discovery of Rock Ptarmigan in eastern Tajikistan (Montgomery & Holder 2020) it remains unclear if the species if present in the mountainous northeastern extremities of Afghanistan, south Kyrgyzstan, northern Pakistan, and Xinjiang. Color wise, the summer plumage of Rock Ptarmigan is grayer than other members of the genus. They also tend to have more slender beaks than other ptarmigan.

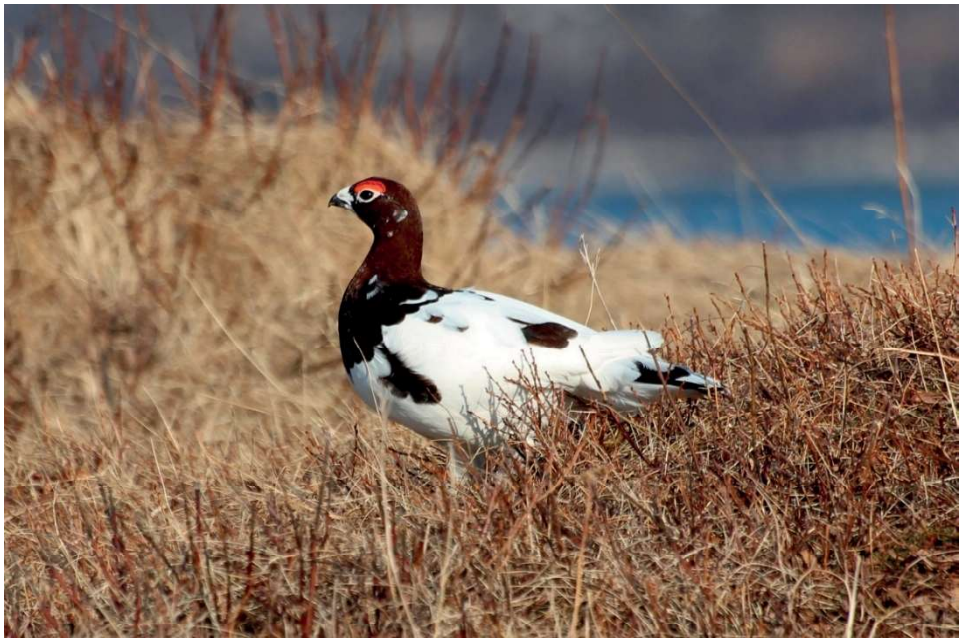

Figure 2. *A spring male Willow Ptarmigan on Store Sommarøya, Norway (Personal Photo / T. Squires).*

1.3.2 Willow Ptarmigan

The Willow Ptarmigan (*Lagopus lagopus*) is found in a wide variety of habitats and is considered an "ecologically plastic grouse" (Hannon *et al.* 2025). In forested areas they have a general preference for dwarf birches (*Betula*) and low willows (*Salix*). The species is estimated to have split with Rock Ptarmigan approximately 2 million years ago (Person's *et al.* 2016). They can be separated from the other species of the genus by males retaining white on the wings in summer plumage and having a more tawny brown coloration outside of winter. Willow Ptarmigan are generally the largest species within the genus.

Figure 3. *A female White-tailed Ptarmigan in Glacier National Park, United States (NPS Photo / G. Eseverri).*

1.3.3 White-tailed Ptarmigan

The White-tailed Ptarmigan (*Lagopus leucura*) is the least studied member of the ptarmigan clade, possessing a smaller global population and more restricted modern range. It appears to represent a more basal lineage of ptarmigan having branched off from ancestors of the other three species approximately 5 million years ago (Persons *et al.* 2016). It can be found in the northwestern portion of North America running from the Pacific Northwestern United States through the Canadian Rockies and up into Alaska. Like the Rock Ptarmigan it is a high-altitude specialist capable of surviving in harsh mountain conditions and appears to alternate between heather communities and alpine pine habitats (Martin *et al.* 2003). It can be distinguished from other ptarmigan most easily by the white tail in all plumages.

Figure 4. *A late winter male Red Grouse in Dumfries & Galloway, Scotland (Public Photo / C. Legg/ CC BY-SA 2.0).*

1.3.4 Red Grouse

The Red Grouse (*Lagopus scotica*) is an aptly named member of the ptarmigan family by virtue of it's appearance. Unlike the other ptarmigan, Red Grouse retain a reddish coloration year round, and never molt into a winter white plumage. Despite this they retain the characteristic third molt found in other ptarmigan (Ahmed *et al.* 2025). It is resident exclusively on the islands of Ireland and Great Britain, along with presence in the Orkney and Shetland Islands. In these areas it is mostly constrained to heather moorlands unlike the closely related Willow Ptarmigan (Ahmed *et al.* 2025). The species is estimated to have diverged from Willow Ptarmigan between 12.5 and 125 thousand years ago (Sangster *et al.* 2022) and retains many shared characteristics.

1.4 Former Genetic Investigations of Ptarmigan

I provide here a broad overview of Ptarmigan genomic investigations, though the provided examples are by no means exhaustive or complete. A significant body of molecular work on ptarmigan already exists with the earliest paper being an investigation of allozyme data for differentiation of Willow Ptarmigan and Rock Ptarmigan populations (Gyllensten *et al.* 1985). A handful of serum esterase loci were used in follow-up studies to investigate the genetics of breeding demography in Willow Ptarmigan (Rørvik & Steen 1989, Rørvik *et al.* 1990). Rock Ptarmigan allozyme variation was used to resolve phylogenetic relationships of the major Phasianidae clades (Randi *et al.* 1991). The genus as a whole is considered monophyletic by all authorities although specific placement still is not ironclad within the family Phasianidae (Luchini *et al.* 2001). Phylogenetic and phylogeographic history of *Lagopus* has recently been updated by the intensive efforts of Persons *et al.* (2016) who incorporated both mitochondrial and autosomal regions to show that the genus likely diverged from a common ancestor to other grouse around 7 to 10 million years ago.

Recent elevation of the Red Grouse to full species status by the International Ornithologists' Union largely relied on genetic information (International Ornithologists' Union 2024, Kozma *et al.* 2019, Sangster *et al.* 2022). The Willow Ptarmigan tends to be the most studied species of the *Lagopus* group and, in general, this is likely due to their greater range at more human-inhabited latitudes and elevations. Many of these studies have focused on the Scandinavian Willow Ptarmigan population (Höglund *et al.* 2013, Berlin *et al.* 2008, Rørvik *et al.* 1999).

Red Grouse have been previously targeted for population studies (Piertney *et al.* 1998, Piertney *et al.* 1999, Piertney *et al.* 2000, McMahon *et al.* 2012) and comparative investigations (Skoglund & Höglund 2010). Authors have also used genetic tools to understand the relationship between Red Grouse of Britain and Ireland and mainland Willow Ptarmigan populations (Quintela *et al.* 2010a, Meyer-Lucht *et al.* 2016) and to address hybridization between Rock and Willow Ptarmigan (Quintela *et al.* 2010b). Beyond breeding ecology and spatial distribution, research on the epigenetic effects of parasite loads has been conducted (Wenzel *et al.* 2014).

Among White-tailed Ptarmigan, genetic research has been sparser and until recently has focused often on populations in British Columbia and Colorado (Fedy *et al.* 2008, Oyler-McCance *et al.* 2010) revealing patterns of island dispersal and long-term regional genetic declines respectively. A paper by Zimmerman *et al.* (2021) looked at genomic differentiation across a major portion of the White-tailed Ptarmigan range.

Genetic investigations of Rock Ptarmigan have centered around investigation of North American, Nordic, and Japanese populations, with only sparse resources available from the Alps, Pyrenees, and Russia, alongside virtually nothing from

populations in Central Asia. The first whole mitochondrial genome sequences were from Iceland, Russia, and Japan (Sveinsdóttir and Magnússon 2017, Wang *et al.* 2017, Yonezawa & Nishibori 2020), though most work relies on microsatellites. Holder and colleagues (2004) disentangled genetic relationships between Rock Ptarmigan in the Aleutian Islands, the North American and Greenlandic Arctic, Iceland, and Newfoundland (Holder *et al.* 2004). That work followed earlier investigations of the glacial refugia hypotheses relying exclusively on the mitochondrial control region (Holder *et al.* 1999).

Rock Ptarmigan in Norway have recently been used to study island biogeography based on the genetic profiles of dispersal to different mountain peaks (Costanzi & Steifetten 2019), while fecal pellets have been assayed for usefulness in non-invasive genotyping (Bergan *et al.* 2016). Previously microsatellites have been used to resolve the colonization routes for Rock Ptarmigan in Svalbard (Sahlman *et al.* 2009). In Alaska, microsatellites have been used to investigate population welfare of a newly translocated evermanni population on the Aleutian island of Agattu (Gregory *et al.* 2011). Previous microsatellite work in the Pyrenees and Alps has focused on dispersal and genetic erosion which is predicted to be problematic in those isolated alpine populations with lower effective population sizes (Caizergues *et al.* 2003). This was expanded with a microsatellite and mitochondrial control region study of the French 'sky islands' shortly after (Bech *et al.* 2009). Genetic sexing of both hunted individuals and feces has established that there are equal adult sex ratios in the French Alps and Pyrenees (Aleix-Mata *et al.* 2020).

Figure 5. *A spring female Rock Ptarmigan in Langanes, Iceland (Personal Photo / Yann Kolbeinsson).*

1.5 Icelandic Ptarmigan, Population Cycling, and Study Areas

The Rock Ptarmigan (*Lagopus muta*), and specifically the Icelandic subspecies *L. m. islandorum*, has long been central to both the ecology and culture of Iceland. Archaeological evidence shows that Vikings arriving during the early settlement period relied heavily on ptarmigan as a food source alongside fish. Middens near Mývatn reveal that ptarmigan were consumed as early as the 9th century, with their eggs supplementing more abundant waterfowl eggs (McGovern *et al.* 2006). This tradition has endured into the modern era, with ptarmigan still appearing on Christmas tables in Iceland (Power 2022, Amilien 2012). For centuries, the bird has been more than just a food source, it has been woven into Icelandic identity and seasonal customs.

The Rock Ptarmigan is the only galliform in Iceland and occupies diverse habitats, including heathlands and grasslands. While sedentary within Iceland, individuals move extensively between breeding and wintering grounds. The local subspecies dominates almost exclusively, and breeds in every corner of the country. Despite this, vagrant Greenlandic *L. m. macruros* have occasionally been recorded (Gudmundsson 1972). The species' diet is well studied: adults feed primarily on birch, crowberry, dryas, and dwarf willow (Dépré & Nielsen 2023), while chicks may incorporate a mixed diet of worms and other invertebrates in spring (Stenkewitz *et al.* 2016), reflecting seasonal variation in forage.

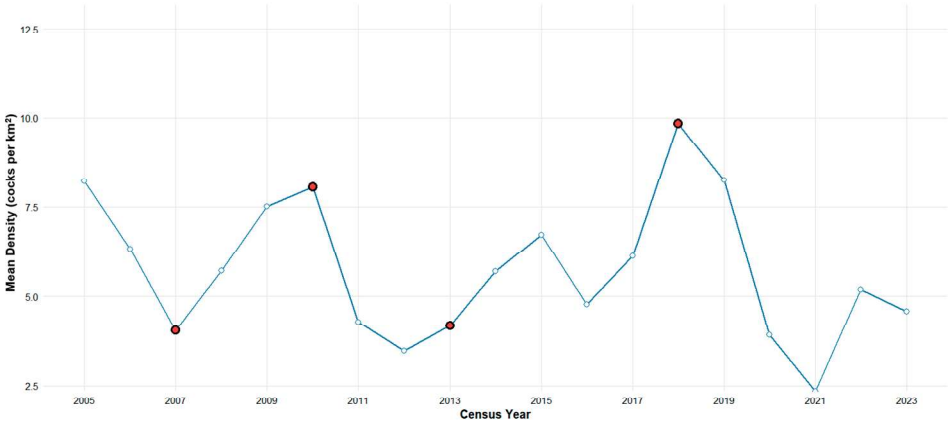

Figure 6. *Mean population densities of male Rock Ptarmigan across Iceland in the last two decades based on May census surveys across six regions (methods detailed in Paper IV, Nielsen 1995). X axis shows census year while Y axis shows average counted ptarmigan cocks per km^2. Red points highlight cohorts utilized in Paper III and IV.*

One of the most striking features of Icelandic ptarmigan populations is their cyclical population dynamics (Fig. 1). Historically, cycles lasted 10–12 years between peaks, but since 2003 they have shortened to about 5 years. These fluctuations are dramatic: modern autumn populations can exceed one million birds in peak years, yet fall below 200,000 in low years (Johnson & Nielsen 2024, Natural Science Institute of Iceland 2025). Such cycles are thought to be driven by food-web interactions, including herbivore–plant dynamics, parasite infestations, and predatory pressures. The Gyrfalcon (*Falco rusticolus*), is a specialized ptarmigan predator (Nielsen & Cade 2017) and a key driver of these cycles (Barraquand & Nielsen 2018). This predator-prey relationship underscores the ptarmigan's ecological importance, and declines in ptarmigan numbers directly threaten gyrfalcon breeding success (Natural Science Institute of Iceland, 2025).

Human exploitation has also shaped ptarmigan populations. Subsistence hunting dates back over 1,100 years (McGovern *et al.* 2006), but commercial hunting emerged in the late 19th century. By the early 20th century, up to 250,000 birds were exported annually to European markets. Exports ceased during World War II, after which domestic consumption dominated (Natural Science Institute of Iceland 2025). In the 1990s, as many as one in four Icelandic households served ptarmigan at Christmas (Power 2022, Amilien 2012). However, heavy hunting pressure contributed to long-term declines, prompting a hunting moratorium in 2003–2004. Since 2005, hunting has been restricted to personal consumption within a defined season, and all sale of ptarmigan has been banned. Today, the species remains listed as 'Near Threatened' on Iceland's Red List (Natural Science

Institute of Iceland 2025; see https://www.natt.is/en/resources/publications/red-lists/red-list-birds/red-list-birds-2025).

Scientific monitoring has been crucial in documenting these trends. Population counts began in the mid-20th century, and long-term monitoring continues today (Natural Science Institute of Iceland 2025). In Northeast Iceland, monitoring efforts including the Rock Ptarmigan Health Project collected hundreds of birds between 2006 and 2018, recording detailed phenotypic data such as body size, organ fat, parasite load, and cecum length (Natural Science Institute of Iceland 2025; see https://www.natt.is/en/research/monitoring-and-research/voktun-rjupnastofnsins) These efforts provide insight into how ptarmigan physiology and health respond to environmental pressures. Such monitoring is vital, as biodiversity declines over the past century have raised concerns about ecosystem stability (Oliver *et al.* 2017). If ptarmigan populations continue to fall, dependent species like the gyrfalcon, and broader Arctic food webs, could be imperiled.

In this thesis I focus on the Northeastern region (*Norðurland eystra*) of Iceland, which is notable for large tracts of high-quality habitat capable of hosting more Rock Ptarmigan than anywhere else in the country (Johnson & Nielsen 2024). In this area, ptarmigan food preferences and diet in the region has been studied since the 1960s (Gardarsson & Moss 1970), and reliable demographic data has been collected since the 1980s (Natural Science Institute of Iceland 2025). Considering abundant phenotypic data and tissues available from the Rock Ptarmigan Health Project, Northeastern Iceland offers an unparalleled opportunity to study the complex life history of local ptarmigan.

1.6 The Genetics of Cycling Populations

Work has been done in recent years to disentangle the effects of high-amplitude cycling on population genetics and today genomic tools can be used to study questions about how allele frequencies behave under these backgrounds. Initial theories were developed by Charles Elton in the early 20th century predicting several outcomes of cyclicity (Elton 1924). Elton proposed that cyclical populations would experience reduced genetic variation, increased differentiation, and that there would be regular temporal shifts in several genetic markers as a result (Elton 1924). While several of his predictions appear to be correct, they remain difficult to test, and a modern consensus is that dispersal and immigration are among the most important factors for the genetics of cycling populations (Norén & Angerbjörn 2014).

Ellner and Hairston (1994) predicted that the “storage of genotypes” due to “generational overlap” from irregular population cycling in any context, could increase the maintenance of diverse alleles. Contrary to initial predictions by Elton it does appear that cycling populations have average or high genetic diversity compared to those with stable demography (Norén & Angerbjörn 2014).

It was recently shown in the Canada Lynx (*Lynx canadensis*) that increasing cycle amplitude reduced genetic diversity and increased genetic differentiation (Row *et al.* 2016) as predicted by Elton. A major finding from this study was that cycling populations experiencing fragmentation, would likely lose diversity and become genetically isolated more quickly than non-cycling populations (Row 2016). There is still much work to be done in understanding the genetic effects of cyclicity across space and time, but it is likely that several trends will be highly specific to local system dynamics. Ultimately, many theories remain untested, primarily due to a lack of reliable temporal data (Norén & Angerbjörn 2014).

1.7 Genetics of a Complex Life History and Chitty's Theory of Polymorphic Behavior

The Rock Ptarmigan's exposure to demographic cycles and various ecological pressures creates a particularly intricate set of trade-offs, which we have started interrogating with state-of-the-art genomic tools. These birds are subject to pronounced population size fluctuations, and the occurrence of these shifts has resulting effects on individual health, survival, and overall fitness. Physiological changes accompany different phases of the cycles, suggesting that individuals are not bound exclusively by external pressures but are also actively responding to shifting ecological contexts (Johnson & Nielsen 2024, Dépré & Nielsen 2023, see also Montgomerie *et al.* 2001). The extent to which these responses are intrinsically genetically wired versus adaptive plastic responses to extrinsic factors has remained unclear. Historically, ecologists have attempted to explain population cycling through either external drivers—such as predation, parasitism, or food limitation—or internal mechanisms, including genetic predispositions and behavioral polymorphisms.

One aspect of my investigations was geared toward understanding the extent to which genetic hardwiring might predispose certain cohorts for survival under set-density conditions. For example, if a trait like rapid fat accumulation is costly, then there should be selection against such a trait when food is readily accessible. Conversely, in periods of scarcity, such a trait might confer a survival advantage. This tension between costs and benefits illustrates the kind of trade-offs that may be central to the persistence of cycling dynamics in ptarmigan populations.

As I introduce in Paper IV, this line of inquiry connects directly to a key hypothesis in ecogenomics developed by Dennis Chitty in the mid 20th century (Chitty 1960, Chitty 1967b). Chitty theorized that spacing behaviors limited a population's density and that these behaviors were genetically derived, capable of responding rapidly to natural selection (Chitty 1977, Krebs 1978). His ideas were originally based on the observation that in cycling vole populations, "individuals in a declining vole population are intrinsically less viable than their predecessors,

and changes in the severity of their external mortality factors are insufficient to account for the increased probability of death" (Chitty 1960). Chitty thus argued that intrinsic changes in the genetic and behavioral composition of populations could drive cycles, rather than cycles being solely the product of external ecological pressures.

This suggestion was, at the time, rejected by followers of Lack (1954), who had argued that food availability was the exclusive driver of population cycles (Chitty 1967a). In a 1978 review of Chitty's theory, Krebs framed the first rule of population regulation as: "No population stops increasing unless the birth rate or death rate is density dependent." Krebs also noted that some components of the environment must prevent population increase, highlighting Nicholson (1933), who suggested that unlimited increases are precluded by parasitism, predation, disease, and food shortages (Krebs, 1978).

In the case of ptarmigan, food availability likely has a limited role in modulating population sizes as density has been shown to respond to land use changes (specifically grazing; Nielsen *et al.* 2004) and preferred forage appears flexible (Dépré & Nielsen 2023). However, parasites and predators are well established to co-cycle with densities. Barraquand & Nielsen (2018), while suggesting that predator–prey feedback was likely responsible for cycling dynamics, could not exclude the possibility that bottom-up drivers were also responsible for fluctuations in ptarmigan population density. In fact, the independent fluctuation of the ptarmigan population might be the predominate driver of Gyrfalcon numbers, with predator dynamics tightly controlled by preferred prey abundance.

Chitty suggested that species could satisfactorily limit their own population densities without the influence of an enemy through density-dependent changes in the quality of individuals (Chitty 1960). The mechanism proposed was rapid changes in the prevalence of traits related to spacing behavior, aggression, and maturation timing. The belief was that selection pressures shift systematically over the course of a cycle, such that traits advantageous during one phase become disadvantageous in another (see also Watson & Moss 1972, Wynne-Edwards 1986). For example, heightened aggressiveness among individuals might limit density during peak phases, while reduced aggressiveness could speed recovery during low phases. This dynamic interplay of behavioral traits could, in theory, generate self-sustaining cycles without requiring external drivers.

Without the genetic aspect, and assuming predator-driven cycling, ptarmigan dynamics can be modeled within the framework of a classic Lotka–Volterra predator–prey system. Yet the possibility of intrinsic behavioral regulation remains compelling. The Red Grouse, for instance, has previously been investigated for relevant trends such as male aggressiveness, which was shown to influence population regulation (Piertney *et al.* 1999, New *et al.* 2019). Such

parallels suggest that behavioral polymorphisms may play a role in other galliform species, including the ptarmigan.

In the present thesis, I do not directly test any of the spatial distribution theories that Chitty suggested. However, I provide some genetic evidence that there may be selection on behavioral traits that could dictate individual fitness and drive population-level trends. These findings hint at the possibility that intrinsic mechanisms (behavior, immune system, etc.), in combination with extrinsic pressures (predation, parasites, etc.), shape the cycling dynamics of ptarmigan populations. Further contributions to Chitty's theory will undoubtedly require many of the same aspects that our current research still needs to grow: larger data sets, integrative modeling approaches, and clearer explanatory pathways linking genotype, phenotype, and population dynamics.

In summary, the Rock Ptarmigan offers a system in which to interrogate the balance between external ecological drivers and internal genetic or behavioral mechanisms.

1.8 Genomic Insights on Local Adaptation

'Local adaptation' refers to the premise of species or individuals being specially tailored for survival in the environments they inhabit. In many respects, local adaptation is conceptually enmeshed in natural selection as survival in given environments is responsible for species persistence and dependent upon adequately selected traits. Without natural selection driving improved adaptation to local environments, the colonization of new habitats and emergent niches would seldom occur. In his book "Adaptation and Natural Selection", George Williams says that the principle of adaptation "should be only used as a last resort" when attempting to explain biological functions (Williams 1966). He also, however, lays the groundwork for understanding how resident genotypes should have higher fitness in given demes than nonresidents (Williams 1966). This ideation explains what is in essence "local adaptation". A branch of biology has centered around the notion that individuals develop improved fitness to ensure survival where they occur.

In local adaptation, there is a significant interplay between natural selection and gene flow that has been widely researched (Kawecki & Ebert 2004). Gene flow is most often thought to reduce local adaptation by maintaining or introducing alleles developed under distant conditions that may be maladaptive in local environments. Selection pushes towards higher fitness in given local environments for niche exploitation, while gene flow serves to homogenize individuals across connected populations and should tend towards general traits. As population sizes increase and especially ranges/exploited habitats expand, species must necessarily become more general and the benefits of local adaptation will decrease in favor of broader success. It is important to note that with adequate

selective pressures locally adapted traits can persist even under high levels of gene flow (Tigano & Friesen 2016). Some genomic features like inversions have also been recognized to act in maintaining differentiation of local populations (Dobzhansky & Sturtevant 1938, Kawecki & Ebert 2004, Jamsandekar *et al.* 2024).

Local adaptation can be considered a major driver of successful adaptive radiations in systems like Darwin's Finches (*Geospiza*) of the Galapagos (Grant & Grant, 2008), and the Honeycreepers of Hawaii (Lerner *et al.* 2011). Genetic architecture in systems like these can be used to track predispositions to adaptive radiation (Rubin *et al.* 2022) or identify the loci selected for adaptive traits (Campana 2019).

Modern genetics provides unprecedented opportunities to quantify the differential success or prevalence of alleles in various environments. Genetic sequencing allows us to track rates of gene flow between populations and infer which isolated groups may be evolving towards different peaks in the fitness landscape (Anderson *et al.* 2010, Johnson 2008). In this way, improvements in genetics have allowed for more clarity in understanding where, when, and how local adaptation is taking place.

It is understood that populations in more extreme habitats or at population edges may be exposed to divergent selection pressures that can drive local adaptation (Kawecki & Ebert 2004). In the ptarmigan, populations like Svalbard have locally adapted to life in the Arctic and experience notable fattening, reduced movement, and metabolic changes to prepare for the winter (Nord *et al.* 2023). The present thesis addresses local adaptation in Paper II, with specific investigation of the genetic adaptations to different regional climates. Furthermore, Paper III and IV seek to understand how a specific population, the Icelandic Rock Ptarmigan, may be genetically handling the local population dynamics which arise in direct response to local ecosystems.

New genomic investigations are contributing regularly to our understanding of local adaptation and evolutionary biology is trending towards integrative approaches. Predictive modelling like our own work in paper III is becoming a major player in revealing how populations may become locally adapted to changing climates (Gain *et al.* 2023). Recent advances in investigating adaptive phenomena will undoubtedly contribute to biology in the context of conservation (Meek *et al.* 2023). The scale at which we can understand adaptive changes over time is also changing as more impressive genomic datasets become easier to generate (Rudman *et al.* 2022). Together these investigations represent the bleeding edge of scientific progress in understanding evolutionary dynamics. Local adaptation provides a foundational framework for understanding evolution, and genomic tools will undoubtedly be an integral part of future investigations.

2 Methods and Findings

2.1 Brief Review of Molecular and Analytical Methods

In all presented studies I utilized Monarch™ Genomic DNA Purification Kits (New England BioLabs, USA) or DNeasy Kits (Qiagen, Germany) for initial DNA extractions. The Reference Genome used in Paper I was sequenced in three parts: Whole genome long-read sequencing of the main sample took place on PacBio Sequel II SMRT Cells at SciLifeLab in Uppsala, while Dovetail Genomics Hi-C Kits were processed on an Illumina NovaSeq 6000 at SciLifeLab in Stockholm. RNA-seq was carried out on Illumina HiSeq2500 system Paired-end 2 × 125 cycles at deCODE genetics, Reykjavík. Each of the samples used in Paper II were sequenced on an Illumina Novaseq 6000 at Uppsala University. Sequencing for the newer 91 Icelandic ptarmigan was performed abroad on an MGI Tech DNBSEQ PE150 paired-end whole genome sequencing device by the Beijing Genomics Institute Group in Hong Kong. Standard bioinformatic cleaning procedures were employed for raw reads including adapter removal with Trimmomatic (Bolger *et al.* 2014), quality checks with FastQC (Andrews 2010), mapping to the reference genome using BWA-mem (Li & Durbin 2009), Evaluation of mapping quality and coverage Qualimap (García-Alcalde *et al.* 2012, Okonechnikov *et al.* 2016), duplicate marking with Picard (http://broadinstitute.github.io/picard/), and variant calling with GATK (Van der Auwera & O'Connor 2020) following best practices. The exact pipelines are outlined in each paper respectively.

Paper I relied heavily on external assembly pipelines developed by the Vertebrate Genome Project (Rhie *et al.* 2021). Annotation of the reference genome relied on the NCBI Eukaryotic Genome Annotation Pipeline version 10.0. My own bioinformatic contributions to this paper focused on annotation and handling of the mitochondrial genome using alignment tools like MitoHiFi (Uliano-Silva *et al.* 2023) and the MITOS WebServer (Bernt *et al.* 2013). The ClustalW package embedded in BioEdit (Thompson *et al.* 1994, Hall 1999) allowed for comparison with previously published ptarmigan mitochondria, while phylogenetic trees were viewed in Mega11 (Tamura *et al.* 2021).

In Paper II, genomic offset was the central method used to assess population vulnerability to climate change. Genomic offset quantifies the mismatch between current allele-environment associations and those projected under future environmental scenarios, providing a value for the genetic change required for populations to remain 'well-adapted'. This approach has recently been applied in conservation genomics to forecast maladaptation risks under rapid environmental change (Capblancq *et al.* 2020, Rellstab *et al.* 2021). By integrating gene–environment association methods from LEA (Frichot & Francois 2015) and

BayPass (Gautier 2015, Olazcuaga *et al.* 2020), with climate projections from CHELSA (Karger *et al.* 2017) and WorldClim (Fick and Hijman, 2017), Paper II demonstrated how offset predictions are highly sensitive to the choice of climate model, underscoring the importance of critically evaluating the environmental inputs in vulnerability assessments.

A central focus of Papers II and III was the quantification of genomic diversity and population structure. Metrics such as nucleotide diversity (π), fixation index (F_{ST}), and absolute divergence (Dxy) were calculated using pixy (Korunes & Samuk 2021), which incorporates invariant sites to provide unbiased estimates. These statistics are standard in population genetics, allowing comparisons of genetic variation within and between populations, and providing insight into the roles of drift, gene flow, and isolation. Importantly, π may be less sensitive to population fluctuations or bottlenecks than F_{ST} and Dxy meaning each metric provides nuanced information about population genetics. In Paper III, allele frequencies were additionally calculated for each sampling year using VCFtools (Danecek *et al.* 2011), enabling temporal analyses of genetic change. To detect evidence of fluctuating selection, allele frequency trajectories were further analyzed using the simulation-based tool varn (Gompert & Messina 2016, Rêgo *et al.* 2019), which accounts for drift and sampling error to identify loci under density-dependent selection.

Paper IV centered on Genome-Wide Association Studies (GWAS) to investigate the genetic architecture of condition-related traits in Icelandic ptarmigan. GWAS is a widely used approach in evolutionary and ecological genetics, associating SNP variation with phenotypic traits to identify loci of potential functional importance (Tam *et al.* 2019, Uffelmann *et al.* 2021). Using GEMMA (Zhou & Stephens 2012), univariate linear mixed models were applied to test associations between ~6 million SNPs and phenotypes such as total fat, lean dry body mass, and composite size metrics. Although no genome-wide significant associations were detected, the results suggested a polygenic architecture, with many loci of small effect contributing to trait variation. Complementary genotype-by-environment (GxE) analyses tested for SNP-by-cycle-phase interactions, further highlighting the complexity of trait–environment relationships in cycling populations.

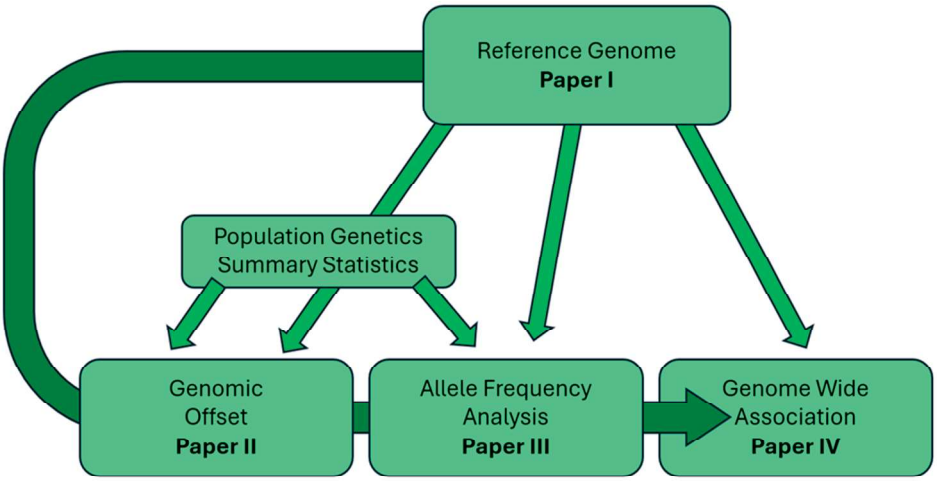

Figure 7*. Flow chart overview of the methods used across the thesis showing the contribution to each paper. A central darker arrow indicates the movement from Paper I through Paper IV.*

The reference genome developed in Paper I directly contributed to all of the subsequent papers. Paper II and III both incorporated investigations of genetic diversity and population structure to inform conclusions (Fig. 2). While Paper II focused on genomic offset, Paper III used allele frequency tracking to draw conclusions. Paper IV analysis roundly relied on GWAS and GxE for exploratory investigations.

Together, these methods form an integrative framework for studying adaptation in natural populations. By combining high-resolution genomic data with ecological and climatic contexts, the thesis advances understanding of how polygenic adaptation, demographic cycling, and environmental change interact to shape the evolutionary trajectories of populations. These methods emphasize both the power and limitations of genomic tools, and particularly highlight the need for careful consideration of climate models, statistical thresholds, and sampling designs in genomic investigations.

2.2 Summary and Findings of Paper I

Whole genome sequencing has become widely accessible over the past decades (Heather & Chain 2016). The first avian reference genomes—Chicken in 2004, Zebra Finch in 2010, and Turkey shortly thereafter—set the stage for a new era of comparative genomics (Warren *et al.* 2010, Dalloul *et al.* 2010). Since then, rapid advancements in sequencing technologies have dramatically expanded the availability of high-quality genetic resources (Heather & Chain 2016). Global initiatives such as B10K and the Earth BioGenome Project have further ensured that avian genomes remain a priority in large-scale sequencing efforts (Zhang

2015, Blaxter *et al.* 2025). Reference genomes allow researchers to compare the evolution of genes across the tree of life in ways that were previously prohibitively expensive and time-consuming. Recent genome-based projects have begun to reshape our understanding of avian phylogenetics and the relationships between bird families (Stiller *et al.* 2024). In this context, the Rock Ptarmigan genome presented in Paper I represents a critical new resource for ecological and evolutionary research.

Reference genomes in combination with population genomics have become instrumental in modern conservation biology, enabling biologically informed decision-making in areas such as translocations, captive breeding, and assessments of regional population health., a well-known example is the Iberian Lynx (*Lynx pardinus*), where genomic resources have been central to recovery efforts (Abascal *et al.* 2016, Kleinman-Ruiz *et al.* 2017, Lucena-Perez *et al.* 2021). Among birds, genome assemblies have supported conservation of endangered species such as the Kākāpō (*Strigops habroptilus*; Dussex *et al.* 2021), the Oriental Stork (*Ciconia boyciana*; Yang *et al.* 2025), and the California Condor (*Gymnogyps californianus*; Robinson *et al.* 2021). Considering the tenuous status of several regional Rock Ptarmigan populations, the availability of a high-quality, annotated reference genome provides a foundation for investigating aspects of biology that are integral to persistence and survival under climate change.

The Rock Ptarmigan genome described in Paper I was developed using PacBio HiFi long-read sequencing and Hi-C scaffolding to capture long-range chromosomal interactions (Squires *et al.* 2023). Long-read sequencing is widely recognized for its ability to reduce gaps in genome assemblies and increasingly provide telomere-to-telomere coverage (Marx 2023). A female bird was sequenced to ensure representation of both Z and W sex chromosomes. The final assembly spans 1.03 Gb, making it relatively small compared to many other bird genomes (Kapusta *et al.* 2017). This compact genome challenges earlier suggestions that less flighted birds tend to have larger genomes (Kapusta *et al.* 2017, Wu *et al.* 2021), and may instead reflect the energetic demands of the harsh environments inhabited by Rock Ptarmigan. The assembly resolved all 40 predicted chromosomes, consistent with the widespread avian karyotype of 2n = 80 (Ohno *et al.* 1964). Annotation predicted just under 20,000 genes, with 16,078 identified as protein-coding (Squires *et al.* 2023). These resources make functional investigation of genetic variation far more tractable. Importantly, the assembly achieved a BUSCO completeness score of 98.6%, placing it among the highest-quality avian genomes to date. In several metrics, the Rock Ptarmigan genome outperforms state-of-the-art chicken assemblies produced by major consortia (Squires *et al.* 2023). This level of quality is particularly notable given that the first “gap-free” human genome was only achieved in 2022 (Nurk *et al.* 2022). While some microchromosomes remain challenging to resolve, the Rock Ptarmigan genome represents a near-complete assembly at the chromosome level.

The Rock Ptarmigan reference genome presented in Paper I represents a formal description of a tool for further ecogenomic studies of Rock Ptarmigan. It provides a foundation for the other studies in this dissertation and has already been adopted by the wider research community (Appenroth *et al.* 2025, Durnova *et al.* 2025). By combining high assembly quality, comprehensive annotation, and public accessibility, this genome positions Rock Ptarmigan as a model for understanding adaptation, conservation, and avian evolution in cold environments. Provisioning this genome was no small feat, but its impact is already evident and will continue to grow as new research builds upon it.

By providing a reliable genomic resource for mapping of genetic variants, Paper I allows for ecogenomic investigations to progress and provides a valuable reference for the genetic analyses performed in the following papers.

2.3 Summary and Findings of Paper II

Ptarmigan are generally sedentary birds, with local populations adapting to the specific conditions of their environments. In Paper II, I investigated patterns of genetic diversity and local adaptation across a significant portion of the species range by analyzing whole-genome resequencing data from 99 individuals sampled throughout Europe, North America, and the Arctic. These genomic data were integrated with environmental variables from two widely used climate datasets, CHELSA and WorldClim, to characterize current genotype–environment associations and to project genomic offset under future climate scenarios. Genomic offset provides a measure of how much genetic change would be required for populations to persist under altered conditions, offering a framework for predicting vulnerability to expected climate change.

A central theme of Paper II is the profound influence of climate data choice on downstream conclusions. Even subtle differences in how temperature or precipitation are represented can propagate into massively divergent predictions of vulnerability. This finding strongly supports the notion that forecasts of climate adaptation are only as reliable as the climate models themselves (see Bobrowski *et al.* 2021). By deliberately comparing how different climate products (WorldClim vs CHELSA), different future scenarios (RCP/SSP pathways), and different association methods (BayPass vs. LEA) can change both which SNPs are flagged and the downstream genomic offset predictions, I demonstrated that while some inferences are robust, others are fragile and highly dependent on methodological choice.

Although the specific candidate SNPs from each test overlapped very little (less than 1% between iterations), their genomic locations often fell within the same gene regions, with over 60% congruence. This suggests that while individual adaptive variants differ between populations, the broader adaptive pathways are conserved. CHELSA tended to produce stronger correlations, BayPass flagged

more variants, and offset scores increased under more severe climate projections. CHELSA and BayPass also produced greater variance in predicted vulnerability, underscoring the sensitivity of results to both climate inputs and statistical methods.

My analyses predicted higher genetic risk for Svalbard and European Alpine populations of Rock Ptarmigan, while Icelandic and Swedish Rock Ptarmigan, along with Red Grouse in Great Britain appeared comparatively less vulnerable. Willow Ptarmigan populations in Norway and Newfoundland also showed elevated offset, largely driven by expected climatic changes. Temperature and precipitation emerged as the strongest predictors of population risk, yet offset predictions differed substantially between climate models. Gene ontology analyses revealed that adaptation was polygenic, with selection acting on different SNPs within a largely conserved set of genes potentially related to neurogenesis and general development. This pattern suggests multiple evolutionary pathways to cold adaptation, consistent with the idea that functional redundancy underpins resilience in these species.

Paper II emphasizes both the promise and the limitations of genomic offset as a conservation tool. While offset maps and risk statements are increasingly presented in the literature, our findings caution against over-interpretation. Predictions depend strongly on climate data choice, scenario selection, and association method, and concordance among multiple approaches is necessary before making strong claims. Genomic offset analyses are most powerful when supported by extensive genetic and climatic resources, and when multiple analytical approaches are tested to achieve consensus.

The main takeaway from Paper II is that consensus models provide the most reliable predictions of genetic vulnerability. Our results demonstrate that accurately forecasting maladaptation to climate change requires not only sophisticated genomic analysis but also a critical appraisal of the foundational climate projections. Conservation planning should therefore prioritize the preservation of functional genetic diversity, which underpins the complex and redundant adaptive responses to a changing world. At the same time, researchers must remain cautious of oversimplification, recognizing that offset results are highly sensitive to methodological choice and should be interpreted responsibly.

2.4 Summary and Findings of Paper III

Icelandic Rock Ptarmigan (*Lagopus muta*) are well established as exhibiting significant population size fluctuations, with census sizes moving from fewer than 260,000 to more than 964,000 individuals nationwide and local densities varying up to 20-fold. These demographic cycles impose density-dependent selection pressures that differ between cohorts born in peak years and those born in trough years. Because phenotypic traits are also known to cycle at the population level

(as later explored in Paper IV), we hypothesized that birds born in peak years versus trough years would carry distinct genetic signatures of selection, reflecting differential survival and reproduction under contrasting ecological conditions.

To test this, I resequenced whole genomes from 91 first-year birds sampled in two trough years (2007, 2013) and two peak years (2010, 2018) from northeastern Iceland, spanning an 11-year study period. I examined changes in genetic diversity, differentiation, and allele frequency shifts across these cycles. Our analyses revealed significant temporal fluctuations in allele frequency patterns. Evidence suggests that a portion of these shifts are derived from short-term fluctuating balancing selection arising from demographic cycling, and we establish that large portions of the genome show higher-than-expected patterns attributable to shifting selection pressures during peak and trough years. Overall, my results suggest that SNPs in Rock Ptarmigan experience fluctuating selection at a significantly higher rate than directional selection, even after accounting for drift.

Balancing selection can take several forms. Heterozygote advantage maintains polymorphism when heterozygotes have higher fitness than either homozygote. Frequency-dependent selection confers an advantage to rare alleles at the expense of common ones. Fluctuating selection in time or space can also preserve variation, with alleles alternating between beneficial and deleterious depending on ecological context (Charlesworth 2006, Hedrick 2006). In the Icelandic ptarmigan, demographic cycling appears to manifest these fluctuating pressures.

From a collection of more than 7 million SNPs we identified 22,399 candidate loci exhibiting significant fluctuating patterns beyond those expected from drift or type one errors. While not all of these SNPs are causal, many are likely linked through linkage disequilibrium to causal variants. Importantly, even modest allele frequency changes (~5%) proved statistically significant after relatively large effective population size estimates (ranging from 897 to 5633 across cohorts) rendered effects from drift small. This sensitivity enabled detection of stronger signals, particularly for loci exhibiting alternating directional changes over time. In general, patterns of allele frequency fluctuations largely mirrored previously reported regional census population sizes (Johnson & Nielsen 2024).

Among the genes repeatedly flagged, *CTNNA2* (Catenin Alpha-2) stood out. This gene, involved in neurological development and startle response, appeared in multiple tests and may plausibly relate to predator avoidance behaviors under density-dependent predation. Other candidate genes included loci associated with immune function, suggesting balancing selection mediated by parasite loads. These findings align with ecological observations that parasite intensity and predator pressure vary with population density, shaping survival and reproduction.

In addition to behavioral pathways that may be influenced during cycling, parasite loads are well established to fluctuate in the Icelandic population (Stenkewitz *et al.* 2016). I found some genes, most notably *GUCY2C* and *TRAF2*

with plausible correlations to handling of parasite infections. *GUCY2* has been previously shown to have a relationship with bacterial infection and gut health (Bose *et al.* 2020) while *TRAF2* is known to regulate cell survival and inflammation (Etemadi *et al.* 2015, Dhillon *et al.* 2019). We suggest that these genes might be specifically naturally selected for their role in immune response to damage from *Eimeria* in the gut.

Paper III therefore demonstrates that large-scale allele frequency shifts in Icelandic Rock Ptarmigan are probably attributable to demographic cycling. Covariance and linkage among sites contribute to broad trends, but the significance of fluctuation across many loci is undeniable. Our results suggest that fluctuating selection is a pervasive force in this system, maintaining genetic variation across the genome. While directional sweeps may occur, particularly under long-term environmental change, fluctuation is the dominant signal over the four time-points observed in our study.

This work highlights the importance of temporal genomics for disentangling the interplay between demography and selection. By capturing allele frequency changes across multiple cycles, we show that balancing mechanisms—whether neurological, behavioral, or immunological—can preserve variation in the face of density-dependent pressures. These findings underscore that demographic cycling is not merely an ecological phenomenon but a powerful evolutionary force shaping the genomic landscape of Icelandic Rock Ptarmigan.

Paper III adds to the central thesis by shifting focus back to the Icelandic Rock Ptarmigan and showing that demographic cycling does in fact appear to influence population genetics changes to a significant degree. We see that there are genes putatively associated with behavior and health that may be cycling in frequency along with population density. Collectively with the putative ontological findings from paper two, we begin building evidence that behavioral adaptations may be a key component of survival.

2.5 Summary and Findings of Paper IV

While the primary application of GWAS remains the identification of causative agents in human disease, its methodological framework has proven equally powerful when applied to ecological genetics and evolutionary biology (Visscher *et al.* 2017). Conservation genetics, however, is not generally able to utilize the full power of GWAS simply due to a lack of genetic resources and low statistical power in tests. Instead, large datasets with adequate phenotype data, and many sequenced individuals are necessary to identify genuine associations. Despite this, GWAS methods are being increasingly used in non-model natural systems such as Atlantic Cod (*Gadus morhua*; Han *et al.* 2025), Atlantic Salmon (*Salmo salar*; Gutiérrez *et al.* 2015), and Eurasian Blackcaps (*Sylvia atricapilla*; Delmore *et al.* 2023), but also in general plant and forest ecology (Meng *et al.* 2025, Roy *et al.*

2025). It seems certain that in the near future, conservation will increasingly benefit from these types of investigation.

In Iceland, ptarmigan populations are well known to fluctuate in multi-annual cycles, with concordant shifts in life-history traits such as morphology, survival, and reproduction. Building on this ecological context, Paper IV explores genotype–phenotype relationships in Icelandic Rock Ptarmigan, focusing on condition-related traits that may vary with demographic cycling. Using whole-genome resequencing and phenotype data from 90 juveniles collected in northeastern Iceland, we performed an exploratory genome-wide association study (GWAS) to assess whether any large-effect loci could be linked to traits such as body size and energetic reserves.

The availability of a specialized dataset from the Icelandic "Ptarmigan Health Study" (Nielsen *et al.* 2014, Natural Science Institute of Iceland 2025) provided a unique opportunity to investigate genotype–phenotype relationships in this system. Although my study ultimately utilized a modest number of genomes, it served as an important exploratory step, allowing for the development of analytical pipelines and the identification of potentially important gene candidates. In the future, larger sequencing efforts and the development of SNP chips will enable rapid genotyping of thousands of birds, facilitating more powerful association tests across regional populations.

Our exploratory GWAS revealed no genome-wide significant associations at conventional thresholds, consistent with the expectation that condition-related traits are highly polygenic. Nevertheless, several candidate loci emerged from reduced tests and targeted analyses. Among these, *HTR1F* (5-Hydroxytryptamine Receptor 1F) was notable. This serotonin receptor gene, previously linked to neurological function and behavior, appeared repeatedly among top hits. Its potential relevance to cognition, memory, and stress response suggests intriguing avenues for future research. Other candidate genes identified in the manuscript—such as those associated with lipid metabolism, body size regulation, and circadian rhythms—provide further hypotheses for investigation.

Several other candidate genes emerged from our exploratory analyses further reinforcing the biological plausibility of our findings. Among top hits from the GWAS on log(TotalFat), *GALNT9* was notable for its prior association with adipose fat deposition in chicken livers (Jin *et al.* 2017), while *ACSL3* and *EBF1* observed in our GxE tests have been linked to lipid metabolism and abdominal fat deposition in poultry (Tian *et al.* 2021, Chao *et al.* 2025).

Paper IV underscores both the promise and limitations of GWAS in conservation genomics. While our dataset lacked the statistical power to detect strong associations, the study highlights the polygenic nature of condition-related traits in Rock Ptarmigan and establishes a foundation for future work. With tissues and phenotype data available from over a thousand individuals, expanded sequencing efforts will allow us to move beyond exploratory analyses toward

robust identification of functional variants. Ultimately, integrating genotype–phenotype associations with ecological context will clarify how ptarmigan populations respond to demographic cycling and environmental change.

Paper IV contributes to the central thesis by connecting observed phenotypic changes known to correlate with population cycle, with genetic signatures putatively indicative of association with the observed traits. This genome-wide association study, though largely exploratory and conducted with low power, appears to add confirmatory evidence to the preliminary findings of Paper III. To this end, new putatively neurological and behavioral genes are identified as possibly associated with fluctuating phenotypes. There is additional evidence that some candidate genes may be fluctuating in response to parasite loads, though additional analysis is needed to confirm this. This suggests that minor-effect alleles may be shifting in frequency to collectively respond to density-dependent selection pressures over time.

3 Main Conclusions

The research presented in this thesis has sought to illuminate the evolutionary and conservation biology of the Rock Ptarmigan (*Lagopus muta*) and its close relatives, with a particular focus on the Icelandic population. Across four papers, I have examined the species from multiple angles: the development of a high-quality reference genome, the assessment of genomic offset under climate change, the detection of fluctuating selection in the context of demographic cycling, and the exploratory investigation of genotype–phenotype associations for condition-related traits. Taken together, these studies provide a multifaceted view of how ptarmigan populations adapt to dynamic environments, how their genetic diversity is structured, and how populations might change in response to density fluctuations.

3.1 Behavioral and Neurological Signatures of Selection

One of the most striking themes to emerge from Papers III and IV is the recurrent involvement of genes associated with behavior and neurological development. I found evidence that alleles important for cognition, stress response, and neural wiring may be differentially selected during demographic cycles. This is particularly compelling in the Icelandic context, where Rock Ptarmigan populations fluctuate dramatically in size due to density-dependent predation by Gyrfalcon (*Falco rusticolus*) and other ecological pressures (Barraquand & Nielsen 2018). It is conceivable that under heightened predation risk, selection favors more conservative foraging strategies and increased fat storage, whereas in years of population growth, when barriers to food are reduced, riskier foraging may be advantageous and fat storage becomes costly. Such dynamics suggest that behavioral wiring itself may be subject to cyclical selection, with alleles influencing risk-taking, startle response, or fat metabolism rising and falling in frequency depending on demographic phase.

The identification of genes such as *HTR1F* (a serotonin receptor linked to cognition and stress response) and *CTNNA2* (Catenin Alpha 2, associated with startle modulation and anxiety-like behaviors) underscores this point. These loci, flagged in Papers III and IV, suggest that neurological pathways may be central to how ptarmigan populations navigate fluctuating ecological conditions. Other candidates, including *GALNT9*, *ACSL3*, and *EBF1*, point to lipid metabolism and fat deposition, reinforcing the idea that energetic reserves are tightly coupled to demographic cycles. The convergence of behavioral and metabolic genes highlights the complex interplay between ecology, physiology, and genetics in shaping survival strategies.

3.2 Fluctuating Selection and Polygenic Architecture

Paper III demonstrated that fluctuating selection is a pervasive force in Icelandic Rock Ptarmigan evolution. We identified over 22,000 SNPs exhibiting oscillating allele-frequency patterns putatively in response to cycling dynamics. Importantly, fluctuating selection appeared to act at a higher rate than directional selection, even after accounting for drift. This finding challenges the traditional view that selection primarily drives alleles toward fixation. Instead, in cyclic populations, selection may maintain polymorphisms by favoring different alleles at different times, thereby preserving genetic diversity.

Paper IV extended this perspective by applying GWAS to condition-related traits such as fat reserves and lean body mass. While no genome-wide significant associations were detected—consistent with the expectation that these traits are highly polygenic—the study identified plausible candidate loci. The absence of large-effect hits reinforces the idea that many genes of small effect shape complex traits in ptarmigan, each contributing incrementally to phenotypic variation. This polygenic architecture is consistent with findings in other natural systems, where traits such as size, fat deposition, and behavior are influenced by networks of genes rather than single loci. Together, Papers III and IV suggest that fluctuating selection and polygenic architecture are complementary forces: cyclical ecological pressures act on a broad genetic substrate, maintaining diversity and shaping trait distributions without driving hard sweeps.

3.3 Conservation Outlook

The potential of population genetics to assist in wildlife conservation and management goals is well recognized (Hohenlohe *et al.* 2021). This thesis provides new context and genomic resources for understanding global populations of birds in the genus *Lagopus* and provides special support for assessment of the previously threatened Icelandic Rock Ptarmigan. Of course, the findings of this thesis are also applicable broadly to understanding evolutionary biology.

My conservation prognosis for Icelandic Rock Ptarmigan is cautiously optimistic. Paper II highlighted that despite a long period of isolation, the birds in Iceland have robust genetic diversity and the predicted environmental changes are less severe than other areas. I expect that if any serious threat may arise in the coming decades it will instead be from new invasive species, or major land use changes related to ecologically uninformed forestry and development. I am hopeful that evidence of relatively good genetic structure and recently improving population trends may indicate that the Rock Ptarmigan is capable of persisting in the face of major ongoing changes to Icelandic ecosystems. Other regional populations such as those of the Alps, Svalbard, and Greenland remain highlighted

for concern due to combinations of genetic and ecological factors. Work will continue to understand adaptation in these populations and determine risks.

Rock Ptarmigan in Iceland are facing an unclear future due to both long term processes and immediate stochasticity in their ecology. Despite being considered threatened with extinction in the last two decades, in 2025 the ptarmigan census population in Iceland reached a 38-year high (Nielsen, Ó.K., *unpublished data*). This rebound is apparently the result of avian influenza infections causing a crash in numbers of predatory Gyrfalcon (Iceland Monitor 2025). Simultaneously, Iceland has just recorded its first purported natural breeding of mosquitos in history (Horton 2025). While these mosquitos might not persist now, rapid environmental changes and new climate stochasticity is likely to present altered ecosystems and new challenges to the Icelandic Rock Ptarmigan population. Such changes could result in new risks from avian malaria or massive changes to vegetation that Ptarmigan rely on for survival.

3.4 Contributions to Ecogenomics

Several major consortiums have been raised in recent years to support sequencing efforts. The Earth BioGenome Project (Lewin *et al.* 2018), Vertebrate Genomes Project (Rhie *et al.* 2021), European Reference Genome Atlas (McCartney *et al.* 2024), and the Bird 10,000 Genomes Project (Feng *et al.* 2020), all strive towards the generation of reference genomes. Paper I contribute to this end by making available a high-quality Rock Ptarmigan reference genome. This new reference genome has already been used in studies of brain activity and circadian biology in the Svalbard Rock Ptarmigan (Appenroth *et al.* 2025), and microbiome work on ptarmigan gut ecology (Durnova *et al.* 2025). Other work that utilized the genome includes investigations of Western Capercaillie and Hazel Grouse karyotypes (Proskuryakova *et al.* 2025), tracking chromosome evolution across vertebrates (Schultz *et al.* 2024), description of the Common Porchard genome (Xia *et al.* 2024), and preparatory work on the Chukar genome (Zhao *et al.* 2023).

Paper II provided comparisons of different climate data bases for calculating genomic offset (see also Hemp & Hemp 2024, Bobrowski *et al.* 2021). This paper makes important insights available regarding the interpretability of offset tests. Furthermore, Paper II identifies putative links between different regional regimes of local genetic adaptation to environments, and the subsequent risk from ecological change. This new case study provides a fundamental example of how genetic welfare of a population may or may not mitigate climate-mediated risks.

It has previously been difficult to reject the possibility of bottom-up drivers of demographic fluctuations (Barraquand & Nielsen 2018). In particular, host–parasite interactions and additional demographic data including genetic information should be considered. Paper III and Paper IV starts investigating genetic aspects of cycling in the Rock Ptarmigan and provides plausible theories

of gene involvement with parasite loads and predator avoidance. Paper III and IV also explore possible genetic effects of cycling (Chitty 1977, see also Krebs 1978). I provide evidence that there are large-scale allelic changes concurrent with population peaks and troughs, and support a key premise that density is putatively tied to selected traits. This suggests that different genes may be fluctuating with density.

3.5 Conclusion and Summary of the Thesis

This thesis brings together several methods of genomic inquiry to examine how ecological forces can have genetic impacts in Rock Ptarmigan (*Lagopus muta*). By leveraging a new reference genome against population-level datasets, with temporal genomic monitoring, the work provides a comprehensive framework for understanding how species respond to both ecological pressures and demography in changing environments.

Paper I established a genomic base to enable mapping, functional annotation, and comparative analyses. Paper II extended this foundation to a broad geographic scale, showing that climate-associated variation in ptarmigan is highly polygenic and that forecasts of genomic vulnerability are strongly dependent on the climate models and statistical approaches employed. Paper III moved towards the influence of temporal dynamics on a population experiencing long-term declines (though it has recently rebounded!), demonstrating that allele frequencies fluctuate with multi-annual demographic cycles. We identified many candidate loci putatively linked to neurological, behavioral, and immune pathways. Paper IV explored the genetics of condition-related traits, finding no large-effect loci but instead evidence for a polygenic traits, while highlighting plausible candidate genes for future investigations.

Taken together, these studies reveal the Rock Ptarmigan as a species subject to multiple, sometimes conflicting, sources of selection. Local adaptation to climate, density-dependent pressures, and condition-related traits all continue interacting to shape its evolutionary trajectory. Our findings underscore the importance of preserving functional genetic diversity, critically evaluating climate projections in conservation planning, and expanding temporal genomic monitoring to capture the dynamics of fluctuating populations.

In conclusion, this dissertation demonstrates how ecogenomic approaches can bridge molecular data with ecological context, providing insights into adaptation and vulnerability in natural populations. While centered on the Rock Ptarmigan, the methods offer a template for studying other species facing environmental change and complex demography in a warming world.

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Genome Report

A chromosome-level genome assembly for the Rock Ptarmigan (*Lagopus muta*)

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Abstract

The Rock Ptarmigan (*Lagopus muta*) is a cold-adapted, largely sedentary, game bird with a Holarctic distribution. The species represents an important example of an organism likely to be affected by ongoing climatic shifts across a disparate range. We provide here a high-quality reference genome and mitogenome for the Rock Ptarmigan assembled from PacBio HiFi and Hi-C sequencing of a female bird from Iceland. The total size of the genome is 1.03 Gb with a scaffold N50 of 71.23 Mb and a contig N50 of 17.91 Mb. The final scaffolds represent all 40 predicted chromosomes, and the mitochondria with a BUSCO score of 98.6%. Gene annotation resulted in 16,078 protein-coding genes out of a total 19,831 predicted (81.08% excluding pseudogenes). The genome included 21.07% repeat sequences, and the average length of genes, exons, and introns were 33605, 394, and 4265 bp, respectively. The availability of a new reference-quality genome will contribute to understanding the Rock Ptarmigan's unique evolutionary history, vulnerability to climate change, and demographic trajectories around the globe while serving as a benchmark for species in the family Phasianidae (order Galliformes).

Keywords: Rock Ptarmigan, *Lagopus muta*, reference genome, PacBio Hifi, evolutionary biology

Significance Statement

The Rock Ptarmigan is a widespread bird species of economic and nutritional importance to large portions of the northern hemisphere. Only a tiny fraction of the Rock Ptarmigan's genome was previously reported and studied. The effort undertaken to sequence and annotate the whole genome provides an ability to understand the species at a molecular level. This vertebrate genome allows for new critical assessment of the Rock Ptarmigan and related species at individual, population, and environmental scales.

Introduction

The Rock Ptarmigan (*Lagopus muta*) is a grouse species with a wide distribution across the arctic and subarctic northern hemisphere. It has seasonally variable plumage ranging from almost entirely white in the winter to heavily mottled gray, rust, and brown in the breeding months (Fig. 1). Birds of the genus *Lagopus* are notable for having feathered legs and feet which likely serve to insulate them in cold habitats. The Rock Ptarmigan can be considered as a ring species with variable genetic diversity across its circumpolar range (Sahlman *et al.* 2009; Kozma *et al.* 2019). Accordingly, Rock Ptarmigan are expected to be at long-term risk across much of their range due to ongoing climatic changes and limited suitable habitat (Costanzi and Steifetten 2019; Masanobu *et al.* 2019).

With the expected declines in cold specialist species as global temperatures rise (Chamberlain *et al.* 2012; Scheffers *et al.* 2016; Scridel *et al.* 2018; Höglund *et al.* 2021), nonmigratory birds are particularly valuable to science as they are likely to display many special adaptations necessary for life in the arctic or at high altitude. Some populations of Rock Ptarmigan are considered near-threatened or endangered due to long-term population loss and expected habitat declines (Icelandic Institute of Natural History 2018; Kozma *et al.* 2018; Japanese Ministry of the Environment 2020). The risks associated with declining genetic quality and environmental changes are not well understood, but might be better assessed with genomic analysis (Bay *et al.* 2018; Formenti *et al.* 2022). For populations with robust historical demographics such as the Icelandic Rock Ptarmigan (Nielsen 1986, 1999, 2011; Nielsen and Pétursson 1995; Brynjarsdóttir *et al.* 2003; Nielsen *et al.* 2004), a locally sourced reference genome is valuable for assessing demographic history.

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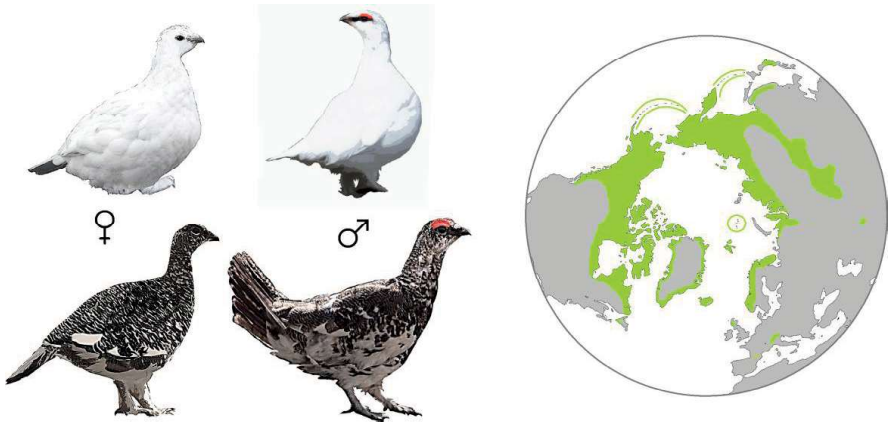

Fig. 1. Species overview showing sexually dimorphic seasonal molt patterns of adult Rock Ptarmigan with white winter plumage and mottled breeding colors, alongside a range map showing the global distribution of Rock Ptarmigan above 30° north.

The species nearest relatives include other grouse in the subfamily Tetraoninae, although systematics in the order Galliformes remain poorly resolved. The mitochondrial genome of Rock Ptarmigan was previously made available along with the mitochondrial DNA of a sister species Willow Grouse (*Lagopus lagopus*; Sveinsdóttir and Magnússon 2017). The Willow Grouse and Rock Ptarmigan are believed to have diverged as recently as 2–5 million years ago (Persons *et al.* 2016) and are often studied together (Lucchini *et al.* 2001; Kozma *et al.* 2018). The white-tailed ptarmigan (*Lagopus leucura*) is the most closely related species with whole genome data available, having a common ancestor with other *Lagopus* taxa no older than 3 million years ago, although the genome assembly is not currently annotated (Clark *et al.* 2016; Kozma *et al.* 2019; GenBank: GCA_019238085.1).

Here, we describe the first reference-quality genome assembly and annotations for Rock Ptarmigan. A combination of long-read and conformation capture sequencing technologies were used to assemble a 1.03 Gb haploid reference genome.

Materials and methods

Sample collection and PCR preparation

As basis for the reference genome assembly and annotation, fresh blood from a single female bird collected (shot) in Húsavík, northern Iceland, in 2018 was used (NCBI BioSample SAMN25144835), while additional, heart, muscle, brain, kidney, liver, ovaries, testes, and spleen from a second bird was collected for RNA-seq to aid in gene prediction (NCBI BioSample SAMN26436951, SAMN29421920, SAMN29421921, SAMN29421922, SAMN29421923, SAMN29421924, SAMN29421925, and SAMN29421926 respectively). DNA extraction was performed in the laboratories of SciLifeLab (Uppsala, Sweden). RNA was isolated, at University of Akureyri, using Beckman Coulter RNAClean XP (FisherScientific, USA). Materials from the birds used for the genome assembly are stored at the Icelandic Institute of Natural History in Garðabær, Iceland (Accession no. RM13211).

Sequencing

Input QC of the DNA was performed using Dropsense, Qubit and Femto pulse to evaluate concentration, purity, and size. The sample library was prepared according to Pacbio's Procedure & Checklist—Preparing HiFi SMRTbell Libraries using the SMRTbell Express Template Prep Kit 2.0. The sample was sheared on Megaruptor 3 with speed setting 30. An Ampure bead purification was performed after the shearing. The samples were size selected using SageElf, according to Pacbio's protocol. Fractions 1 was used for sequencing. Quality control of sheared DNA and SMRTbell libraries was performed on Fragment analyzer, using the Large Fragment standard sensitivity 492 kit. Primer annealing and polymerase binding was performed using the Sequel II binding kit 2.0. The sample was sequenced on the Sequel II instrument, using the Sequel II sequencing plate 2.0 and the Sequel II SMRT Cell 8M, movie time 30 h and pre-extension time 2 h. Whole genome sequencing was carried out at SciLifeLab in Uppsala, while Dovetail Genomics Hi-C Kits were processed on an Illumina NovaSeq 6000 at SciLifeLab in Stockholm. RNA-seq was carried out on Illumina HiSeq2500 system Paired-end 2 × 125 cycles at deCODE genetics, Reykjavík.

Genome assembly

The genome was assembled following the Vertebrate Genome Project (VGP; Rhie *et al.* 2021) assembly pipeline. First, a kmer database was generated using Meryl (v. 1.3) from the PacBio HiFi reads for reference-free genome evaluation and downstream assembly QC. The kmer size was set to 21 after running the best_k.sh script for the expected genome size (~1 Gb) in Merqury (v. 1.3; Rhie *et al.* 2020). PacBio HiFi reads were assembled using hifiasm (v. 0.15.1-r334; Cheng *et al.* 2021), followed by a round of purge_dups (v. 1.2.5; Guan *et al.* 2020) incorporating minimap2 (v. 2.17-r941). Each of the previous steps was followed by assembly evaluation. This included contig/scaffold statistics computed using the Python library assembly_stats (v. 0.1.4), and BUSCO (v. 5.3.1), while completeness and quality value statistics of the assembly along with kmer spectrum plots were produced using Merqury (v. 1.3; Rhie *et al.* 2020). The assembly was scaffolded using the Hi-C reads. Briefly, reads were first aligned to the assembly using the VGP modified version of the Arima mapping pipeline that uses bwa mem (v. 0.7.17-r1188) and samtools (v. 1.19) for alignment and Picard (v. 2.10.3) for 5´end filtering and duplication removal. Scaffolding was performed using Salsa2 (v. 2.3) and evaluated using BUSCO and scaffold statistics.

The Hi-C reads were then mapped back to the scaffolded assembly using the same pipeline as in the previous step and the resulting bam file was converted to pretext format using PretextMap (v. 0.1.7).

The finalized assembly was screened for contamination and then manually curated (Howe *et al.* 2021). Curation was performed using gEVAL (Chow *et al.* 2016) and Hi-C contact maps visualized in HiGlass (Kerpedjiev *et al.* 2018) and PretextView (v. 0.2.5; see Fig. 2), resulting in 97 missed or mis-join corrections to the scaffolds producing a resolved chromosome level genome with 38 autosomes and, the Z and W sex chromosomes. Construction of microchromosomes was investigated using the Mummer alignment tool (v 4.0.0: Marçais *et al.* 2018), although poor syntany was noted for comparison with *Gallus gallus* and some likely remain unresolved.

The mitochondrial genome was assembled separately from both raw reads and contigs using MitoHifi (v. 2.2; Uliano-Silva *et al.* 2021) with automatic alignment to the Japanese Rock Ptarmigan (*L. muta japonica*; Yonezawa and Nishibori 2020) via built-in features from the MitoFinder dependency (v. 1.4.1; Allio *et al.* 2020).

The completed genome assembly is publicly available in NCBI under accession number GCA_023343835.1. The mitochondrial assembly is publicly available in NCBI under accession number OQ580988.

Genome annotation

The Rock Ptarmigan reference genome was annotated using the standard NCBI Eukaryotic Genome Annotation Pipeline version 10.0. A detailed summary of the pipeline is available online at: https://www.ncbi.nlm.nih.gov/genome/annotation_euk/process/. In contrast to previous iterations, this version of the pipeline used RFAM (v. 14.6; Kalvari *et al.* 2021) for discovery of small noncoding RNA's and STAR (Dobin *et al.* 2013) for alignment of RNA-seq reads from our supplementary tissues. The pipeline has stable use of several tools including BUSCO (v. 4.1.4; Manni *et al.* 2021) and Splign (Kapustin *et al.* 2008) among others.

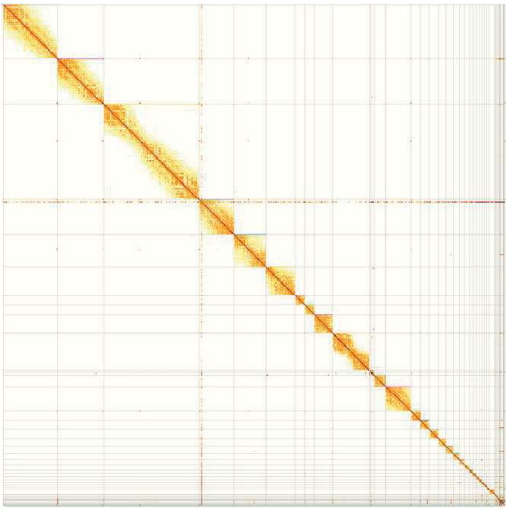

Fig. 2. Hi-C contact map for the bLagMut1 genome showing long-range contacts generated using PretextView (v. 0.2.5).

For calculation of genomic masking, the Rock Ptarmigan genome was masked with WindowMasker (Morgulis *et al.* 2006). Annotation of the mitochondrial genome was achieved via manual comparison with the extant published Icelandic Rock Ptarmigan mitogenome in addition to automatic annotation using MITOS WebServer (Bernt *et al.* 2013).

Results

Sequencing and assembly results

The final assembly sequence is 1,026,771,810 base pairs long, with 71,937 gap bases (0.007%) across 210 spanned gaps (Fig. 3). The genome assembly includes 375 contigs arranged on 165 scaffolds. The scaffold N50 is 71,229,700 bp with an L50 of 5. The Contig N50 is 17,905,263 bp with an L50 of 19.

Average coverage across the genome is 57.75×. In total 38 autosomes were identified, with 18 unlocalized sequences among them. Additional W and Z allosomes were described with only a single unlocalized sequence found on the W. Assembly summary statistics appear significantly better than the current *G. gallus* reference genome (GRCg6a), and are modest in comparison to the most recently annotated *G. gallus* individual (bGalGal1.mat.broiler.GRCg7b; see Table 1). Kmer spectra plots overall showed the expected copy-kmer distributions (Fig. 4).

Genome annotation

In total 20,110 genes and pseudogenes were identified by combining gene prediction and similarity approaches, with approximately 80% identified as protein coding. The annotated genes showed a 98.6% completeness score against 98.9% for the whole genome when set against the BUSCO avian dataset (aves_odb10 lineage) and indicating 0.9% of genes missing from the annotated assembly. The annotation and associate summary statistics are available in NCBI's RefSeq genome record for the reference (Pruitt *et al.* 2013). The contents of the report are summarized in Table 2.

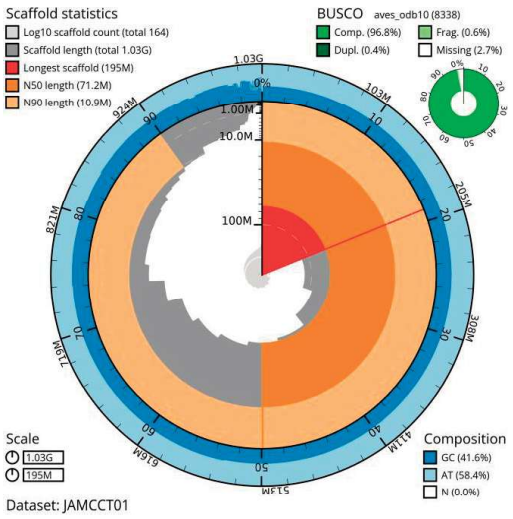

Fig. 3. A snail plot indicating the completeness of the bLagMut1 genome assembly. Summary information about scaffold statistics, BUSCO, and the Guanine-Cytosine vs Adenine-Thymine composition of various regions are included.

Table 1. A series of "global" statistics published in the public release of the Rock Ptarmigan reference genome on NCBI indicating the completeness of the new reference genome in comparison to the gold standard Chicken reference genome and the most recently annotated Chicken reference genome.

	Lagopus muta Reference Genome (bLagMut1)	*Gallus gallus* Most Recent Annotation (bGalGal1.mat.broiler.GRCg7b)	*Gallus gallus* Reference Genome (GRCg6a)
Total length	1,026,771,810	1,053,332,251	1,065,348,650
Total ungapped length	1,026,699,873	1,049,948,333	1,055,564,190
Gaps between scaffolds	0	0	68
Number of scaffolds	165	214	524
Scaffold N50	71,229,700	90,861,225	20,785,086
Scaffold L50	5	4	12
Number of contigs	375	677	1,402
Contig N50	17,905,263	18,834,961	17,655,422
Contig L50	19	18	19
Chromosomes and plasmids	41	42	34
Component sequences	165	677	2,243

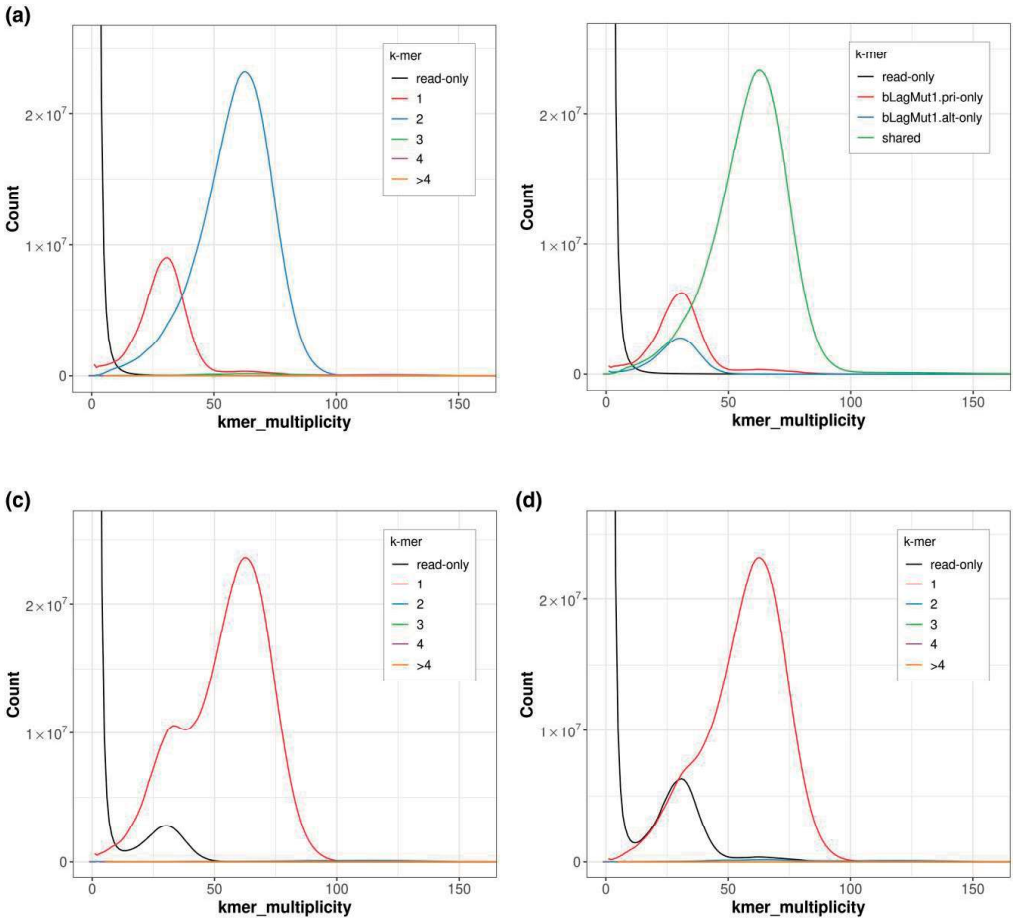

Fig. 4. Outputs from Merqury showing kmer distribution according to: a) Spectra-cn plot of the bLagMut1 complete assembly, b) spectra-asm plot of the bLagMut1 complete assembly, c) spectra-cn plot of the bLagMut1 primary assembly, and d) spectra-cn plot of the bLagMut1 alternate assembly.

Table 2. Comparative table showing the relative accuracy and completeness of the *Lagopus muta* reference annotation (NCBI *Lagopus muta* Annotation Release 100) against the most recently complete annotation of the chicken genome (NCBI *Gallus gallus* Annotation Release 106).

	Lagopus muta Reference Genome Annotation (bLagMut1)	*Gallus gallus* Most Recent Annotation (bGalGal1.mat.broiler.GRCg7b)
Genes and pseudogenes	20,110	25,635
Protein-coding genes	16,078	18,023
Noncoding genes	3,738	7,330
mRNA	43,785	68,670
Long non coding RNAs	5,431	10,062
tRNA	306	303
Protein coding sequences	43,793	68,683
Introns (mean length)	206,142 (4,265)	241,290 (4,145)
Exons (mean length)	229,018 (394)	262,919 (490)
Mean gene size	33 kb	28 kb
Maximum gene size	1.6 Mb	1.3 Mb
BUSCO score	98.6%	98.7%

Mitochondrial genome

The mitochondrial DNA was described with all 13 expected protein-coding regions and analyzed for accuracy through comparative analysis. With our addition, there are now four extant mitochondrial genomes published for the Rock Ptarmigan; two from Iceland, one from Japan, and one from Siberia (Sveinsdóttir and Magnússon 2017; Wang *et al.* 2017; Yonezawa and Nishibori 2020). Using the ClustalW package embedded in BioEdit (Thompson *et al.* 1994; Hall 1999), we found a total of 24 bases divergent from the previously published Icelandic Rock Ptarmigan mitogenome in a manual review. Of these divergences, 14 appeared in coding regions and 8 appeared unique to the previously published individual and our calls at those locations were conserved in the other Rock Ptarmigan populations. None of the polymorphisms observed between the populations appeared to be uniquely conserved in the Icelandic Population. Analysis of pairwise distances using phylogenetic tree software in Mega11 (Tamura *et al.* 2021) showed clear grouping of the Rock Ptarmigan separated from the Willow Ptarmigan, as previously reported (Sveinsdóttir and Magnússon 2017).

Discussion/Conclusion

Our avian reference genome includes a highly complete set of information with 99.994% of the 1.03 Gb described matching to 40 haploid chromosomes and the mitochondria. Other recent works have aimed to unlock the potential provided by Rock Ptarmigan genetics (Kozma *et al.* 2018; Kozma *et al.* 2019; Sigmarsdóttir 2022). As observed for other recently published genomes (Formenti *et al.* 2022), the new Rock Ptarmigan genome is of comparatively excellent quality (see also Table 1).

Although Rock Ptarmigan has been globally identified as Least Concern by the IUCN in recent years, there have been regional fluctuations in its status and some nations identify the species as threatened due to long-term declines (Icelandic Institute of Natural History 2018; European Commission 2022; IUCN 2023). There is evidence that subpopulations of other grouse species may poses important local adaptations necessary for persistence (Oh *et al.* 2019), making it probable that the Rock Ptarmigan has unique evolutionary adaptations across its range. Further, it is well established that Arctic species such as Rock Ptarmigan may be disproportionately affected by climate change with an expected poleward contraction of species' ranges (Birdlife International 2015; Kozma *et al.* 2018). For more disparate populations such as those in the Japanese mountains of Honshu, the European Alps, and the Pyrenees, rising tree lines may entirely squeeze the Rock Ptarmigan out of its montane niches as has been suggested broadly for alpine habitats (Dirnböck *et al.* 2011; Mountain Research Initiative EDW Working Group 2015), and some closely related species (Jackson *et al.* 2015). In the context of conservation, having a reference genome available will contribute to our understanding of the species' genetic risks and possible movements in the face of a warming planet (Bay *et al.* 2018; Kozma *et al.* 2018).

Many wildlife species are difficult to study at the genomic level due to limited specimen availability and constraints on procurement (Kemp 2015; Hope *et al.* 2018). Because the Rock Ptarmigan is a widespread game bird, it is particularly useful for both genomic studies and general investigations into wildlife ecology. Hunters have the potential to contribute robust data regarding the species trends and may continue to contribute both historical and new specimen materials for research (Cretois *et al.* 2020). Given the species' close cultural connection to some regions and history as a food source (McGovern *et al.* 2006), the Rock Ptarmigan may benefit from additional conservation efforts from an involved public or concerned hunters and may be a good candidate for flagship status (McGowan *et al.* 2020).

Future studies into Rock Ptarmigan genomics will benefit from decades of studies into these birds in captivity (Stokkan *et al.* 1988). Recently, Rock Ptarmigan hatched and raised in captivity have been used for gene expression studies to understand circadian rhythms and investigate the cecal microbiome representing valuable opportunities going forward (Salgado-Flores *et al.* 2019; Appenroth *et al.* 2020; Appenroth *et al.* 2021).

Among avian diversity, the birds in the family Galliformes represent less than 3% of all species but have an outsized impact on global economics with Chickens, Turkeys, Pheasants, Quails, and Grouse all being regularly consumed. Among the available avian genomes (Bravo *et al.* 2021) those in order Galliformes are represented with 26 species assemblies currently available on NCBI (approximately 5% of all extant; Sayers *et al.* 2022). Among these, 68 assemblies have been completed and the chicken has been assembled 30 times (for context see Burt 2005; Li *et al.* 2022). This highlights a commercial implication for Rock Ptarmigans as they have many special adaptations that could be of importance to domestic poultry.

Given the usefulness of wild relatives for research into domesticated species (Li *et al.* 2020; Jackson *et al.* 2015) the Rock

Ptarmigan may prove to be a useful model for understanding other Galliformes. This relationship will surely have limitations in the genomic realm as more distantly related species are less informative at finer scales than those that are closely related (Scutari *et al.* 2016). However, if the Rock Ptarmigan's genes tailored to arctic landscapes can be used to better understand genetic architecture for cold weather survival, improved forage capabilities, or other ancestral traits, then important pathways may be identified for commercially exploited birds or other species of conservation interest.

Taking all of this into consideration, the availability of a Rock Ptarmigan reference genome makes the species exceptionally well positioned for investigation across a broad new range of scientific inquiry. With links to arctic/alpine biomes, conservation, hunting culture, and industry, the Rock Ptarmigan reference genome provides a unique opportunity to investigate a species at the intersection of many issues of global significance.

Data availability

The final annotation has been publicly released and uploaded according to the high standards of the Earth BioGenome Project (Lewin *et al.* 2018). The genome assembly, including the raw shotgun sequencing data, has been uploaded to NCBI and is available at https://www.ncbi.nlm.nih.gov/assembly/GCA_023343835.1; BioProject: PRJNA836583; BioSample: SAMN25144835. The mitochondrial assembly is publicly available in NCBI and is available at https://www.ncbi.nlm.nih.gov/nuccore/OQ580988.1.

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Conflicts of interest statement

The author(s) declare no conflict of interest.

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Paper II [redacted]

Genomic vulnerability in Arctic birds is driven by polygenic adaptation and confounded by climate model choice

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Data Availability

Upon acceptance of the manuscript, raw sequencing data will be uploaded on SRA. Output files will be uploaded on Dryad (10.5061/dryad.crjdfn3j) and analysis scripts will be made available on Dryad and PRMs/TESs GitHub pages.

Abstract

Predicting species resilience to climate change is a primary goal in conservation, yet such forecasts are often hampered by uncertainty in both genomic and environmental data. For cold-adapted species in rapidly warming regions like the Arctic, understanding these uncertainties is crucial for effective management.

Here, we use 99 whole-genomes of rock ptarmigan (*Lagopus muta*), willow ptarmigan (*L. lagopus*), and red grouse (*L. scotica*) from 12 populations across the North Atlantic to identify patterns of genetic diversity, local adaptation, and predict genomic offset under future climate scenarios. To assess this, we integrated these data with two leading global climate models (CHELSA and WorldClim) and two complementary gene-environment association methods (BayPass and LFMM).

Our analyses reveal that predictions of genomic vulnerability are highly sensitive to the choice of underlying climate data, leading to conflicting conclusions regarding conservation priorities for populations in Greenland, Svalbard, and the Alps. We identify widespread, polygenic adaptation to climate, with selection acting on different SNPs within a largely conserved set of genes related to neurogenesis and general development, suggesting multiple evolutionary pathways to cold adaptation. Genomic diversity was lowest in the most isolated populations (Svalbard, Pyrenees, Iceland), consistent with patterns of genetic drift though this did not correlate directly to calculated offset.

Our findings demonstrate that accurately forecasting maladaptation to climate change requires not only sophisticated genomic analysis but also a critical appraisal of the foundational climate projections. The choice of climate data can be as influential as the biological signal itself, a crucial consideration for all vulnerability assessments. We conclude that conservation efforts for these species should prioritize the preservation of functional genetic diversity, which underpins the complex and likely redundant adaptive responses to a changing world.

Introduction

Anthropogenic climate change is one of the most pressing threats to wildlife populations worldwide (Parmesan 2006; Scheffers et al. 2016). Average global temperatures have been rising and are expected to drastically increase in the immediate future (Collins et al. 2013; CAMS 2024). Populations and species can respond to climate change in three essential ways. First, species may track the change by altering their distribution. Second, they may remain in the same place and adapt to the new circumstances via genetic changes, which necessitates that standing genetic variation is available within the population, and/or adequate phenotypic plasticity is present. Third, if neither of the above is possible the populations of such species ultimately go extinct (Parmesan & Yohe 2003, Pinsky et al. 2013, Urban 2015, Höglund et al. 2020). Directional environmental shifts such as increased temperatures or decreased rainfall often have limited direct effects on species but may instead cause more compounded effects mediated by ecosystem change such as altered community structure (Cahill et al. 2013).

Levels of genetic diversity vary within and among individuals of any species, and it is unclear how much and what kind of genetic variation is needed for adaptation to changing environments (Rodrigues & Congi, 2021). Connectivity among sub-populations plays a crucial role (Lamarque et al. 2013). While dispersal among sub-populations may mitigate the effects of global climate change by increasing genetic diversity (Aitken et al. 2008), local adaptation could be counteracted by gene flow if there is outbreeding depression as a result of introducing locally maladapted genes (Aitken & Whitlock 2013). It is also possible that species have become so specialized to a given ecological niche that they cannot adapt to new circumstances, especially when environmental changes are rapid (Roman-Palacios & Wiens, 2020). Thus, a pressing evolutionary question within contemporary conservation science is: do populations go extinct because of low genetic variation and inbreeding problems, or are they driven to extinction before any loss of genetic variation has impacted them, either by stochastic effects or vulnerable life histories (Höglund 2009, Frankham et al. 2010, Allendorf et al. 2022). Here genomic approaches and species occurrence and climate data offer a way to study and predict the genetic and evolutionary effects of climate change (Franks & Hoffman 2012, Stillman & Armstrong 2015, Alhajeri & Fourcade 2019, Hoffman et al. 2021, Lajeunesse & Fourcade 2022).

Calculating genomic offset is a promising new tool for estimating how maladapted a given population is in relation to anticipated environmental changes (Lind et al. 2024). This process involves analyzing genetic correlations with current environmental conditions and contrasting them with predicted future genotype-environment correlations. In this way, one can determine if a population will require larger or smaller genetic changes to meet the adaptive demands of a changed environment. Assuming populations are well adapted for their currently occupied niche, those exhibiting lower genetic diversity and experiencing larger environmental changes will show a greater genomic offset, thereby increasing their risk of decline or extinction (Capblancq et al. 2020, Rellstab et al. 2021). However, this approach relies on key assumptions, including that populations are at an adaptive equilibrium and that spatial climate gradients are effective proxies for future temporal change, which may not always hold true in natural systems.

In this paper, we address the relationships between genetic diversity, geographical separation and the environment by studying three closely related sedentary bird species from varying climatic and

demographic backgrounds. The rock ptarmigan (*Lagopus muta*), willow ptarmigan (*L. lagopus*), and red grouse (*L. scotica*) diverged in the last 2 million years (Persons et al. 2016) and form a tight species complex exhibiting similar plumages, behaviors, niche overlap, and genomic architecture (Kozma et al. 2019). While each species is naturally distributed within the Holarctic and shows cold-weather adaptations (Höglund et al. 2013; Holder et al. 1999), the rock ptarmigan remains the most cold adapted of the three and occurs in arctic and sub-arctic regions whereas willow ptarmigan and red grouse occur in more open sub-alpine habitats, boreal forests, and moorlands at lower latitudes (Watson et al. 1998; Storch 2000, 2006; Lucchini et al. 2001).

Using whole genome re-sequencing data from 99 individuals, we focus on population-level differences associated with climate and consider genomic variation across the North Atlantic. By examining regional populations to find genetic associations with the environment we aim to: (1) Quantify and compare patterns of genomic diversity across the species complex, testing the hypothesis that more isolated populations exhibit lower diversity; (2) Identify the drivers and genetic architecture of local climate adaptation by contrasting two leading gene-environment association methods; and (3) Assess the vulnerability of populations to future climate change using the genomic offset framework, critically evaluating how predictions are impacted by the choice of climate data (CHELSA vs. WorldClim) and association method (LEA vs. BayPass).

Methods

Whole-genome re-sequencing, read mapping and variant calling

Tissue samples of individual rock ptarmigan were collected from the Pyrenees (France, n = 10), the Alps (France, n = 8), the eastern (n = 9) and the western coast of Greenland (n = 10), Iceland (n = 10), Sweden (n = 6), and Svalbard (n = 8). Willow ptarmigan samples came from Newfoundland (Canada, n = 6), Sweden (n=6), and Norway (n = 9). Red grouse samples came from northern England (n = 9) and Ireland (n = 6; all shown in Figure 1). Until recently the red grouse was considered a subspecies of willow ptarmigan, but recognition as a separate species is now suggested (Sangster et al. 2022). For most analysis we treat red grouse and willow ptarmigan as one group and rock ptarmigan as another to compare their general life histories, while acknowledging the proposed taxonomic split. The sequences from Ireland were obtained by Walsh et al. (2024), and those from Norway and England were obtained from Kozma et al. (2019). All the other samples are new to this study. The total of 99 samples originated from legally hunted birds (wing muscle tissue) with exception of samples from Svalbard (blood samples) which originated from a colony of first-generation captive birds (for detailed sample information including geographical coordinates, see Table S1). Samples were transferred to Uppsala University or University of Akureyri in ethanol or lysis buffer and kept at 4°C before DNA extraction. Most of the DNA was extracted using a high salt extraction precipitation protocol with an extra ethanol precipitation step (modified from Paxton et al. 1996), while Icelandic and eastern Greenlandic rock ptarmigan DNA was extracted using a Monarch™ Genomic DNA Purification Kits (New England BioLabs, MA, USA). DNA was checked for concentration and purity using a NanoDrop® 2000 spectrophotometer and Qubit® 3.0 fluorometer Quantitation Kit (Invitrogen™, Thermo Fisher, Waltham, MA, USA). Libraries were prepared

using the TruSeq Nano DNA library preparation kit and sequenced on a S4 flowcell of Illumina Novaseq 6000 by SNP&SEQ technology platform (Illumina, CA, USA) at Uppsala University.

We removed adapters from raw reads using Trimmomatic v.0.36 (Bolger et al. 2014) and evaluated the quality of the reads using FastQC v. 0.11.9 (Andrews 2010). We then mapped trimmed reads onto our assembled chromosome level rock ptarmigan reference genome (bLagMut1; Squires et al. 2023; GenBank accession: GCA_023343835.1) using BWA-mem v.0.7.17 (Li & Durbin 2009). We assessed mapping quality and average read coverage using Qualimap v.2.2.1 (García-Alcalde et al. 2012; Okonechnikov et al. 2016), sorted bam files, and marked read duplicates using Picard v.2.20.4 (http://broadinstitute.github.io/picard). In order to decrease variance in sample coverage and facilitate downstream depth filtering across samples, we down-sampled high-coverage samples to ~35X using SAMtools v.1.10 (Li et al. 2009). We used GATK v.4.1.1.0 (McKenna et al. 2010) and best practices for variant calling (DePristo et al. 2011; Van der Auwera & O'Connor 2020). First, we ran HaplotypeCaller with -ERC GVCF enabled on the duplicate marked and down-sampled bam files, followed by combining gVCFs for each individual and chromosome using CombineGVCFs, and then jointly genotyped using GenotypeGVCFs. From the subsequent variant call file with genotyped variants, we separately selected biallelic SNPs and INDELS using SelectVariants. We filtered the resulting VCFs using "hard filtering" standards for the SNP and INDEL variants. First, filtering out SNPs with Phred quality (QUAL) < 30.0, root mean square mapping quality (MQ) < 40.00, strand odds ratio (SOR) > 4.00, variant quality by depth (QD) < 2, fisher strand bias (FS) > 60.00, mapping quality rank sum test (MQRankSum) < −12.5 and read position rank sum test (ReadPosRankSym) < −8.00. For INDELs we used (QUAL) < 30.0, MQ < 40.00, SOR > 10.00, QD < 2.00, FS > 200.00 and ReadPosRankSum < −20.00. We filtered the final VCF by read depth (15 < DP < 65), allowing a maximum of 10% missing data across samples using vcftools v.0.1.15 (Danecek et al. 2011). For final analysis of variants between the species we removed all sites with missing data and pruned all SNPs in a pair with an LD score of 0.75 or higher in 10kb windows. The final VCF for all individuals combined was used in main analyses and included 6,268,635 variants after pruning, while reduced variant files for the *L. lagopus/scotica* group had 4,973,265 SNPS, and *L. muta* had 4,702,905 after removal of invariant sites. A post-hoc analysis of sequence data showed no evidence of batch effects as a consequence of data originating from different Illumina platforms and machines (Figure S1).

Genomic Diversity, and Population Structure

We used the package 'LEA' v.3.12.2 (Frichot & Francois 2015) in R version 4.3.1 (R Core Team 2023) to estimate ancestry coefficients and test fits across several *K* values to determine the best model of observed population structure, using the sparse non-negative matrix factorization (snmf) function in LEA and its associated cross-entropy criterion. According to the "elbow" method based on a Tracy-Widom test the optimal number of ancestral populations was suggested to be between *K* = 10-12 (Figure S2).

To complement snmf we also used ADMIXTURE v.1.3.0 (Alexander et al. 2009) to estimate ancestry proportions across a range of K values allowing us to further explore biologically relevant levels of population sub-structure. K=10 was chosen because it provided the most biologically interpretable clustering that aligned with known geographic and taxonomic boundaries. We present the results for alternative *K* values in the supplement (See figures S3 - S6).

We included 12 populations in our original sampling and calculated autosomal nucleotide diversity (π), absolute divergence (D_{XY}), and the fixation index (F_{ST}) within those predetermined populations using pixy v.0.95.0 (Korunes & Samuk 2021). We choose a standard window size of 10kb and averaged each summary statistic across windows. We ran subsequent tests for all birds at K=10 based on the "cleanest" delineation of observed ancestry proportions based on LEA and ADMIXTURE (Figure 2; see population structure results). For each reduced species group test we accordingly used K=6 for *L. muta* and K=4 for *L. lagopus/scotica*.

Selection of Climate Data

We used two popular global climate datasets, "Climatologies at High Resolution for the Earth's Land Surface Areas" (Hereafter, CHELSA; Karger et al. 2017), and "WorldClim" (Fick and Hijman, 2017) to infer large scale geographical adaptation and estimate the offset to future climate change. Each dataset has informed ecological studies in the recent past (Kozma et al. 2018, Bjorkman et al. 2018, Carlson et al. 2022, Brauer et al. 2023, Pearman et al. 2024), though only a few direct comparisons between models have been made (Karger et al. 2021; Bobrowski et al. 2021). While each dataset provides information on bioclimatic variables, the present climate is determined from historical information and future climate is determined by projection modeling under varying assumptions of greenhouse gas emissions and socioeconomic development (as clarified in Meinshausen et al. 2020). In CHELSA, the present climate is a collection of points determined by global weather data gathered from stations between 1981 and 2010, whereas in WorldClim this historical data was collected in a similar fashion between 1979 and 2013. To ensure comparable inputs for future climate calculations we chose to use sets informed by the Australian Community Climate and earth system simulator (ACCESS) for both sets (Ackerly & Dommenget 2016). Chelsa and WorldClim are both able to incorporate the ACCESS model and make predictions for the future window of 2060 to 2080, but in different ways. CHELSA makes ACCESS1.0, available at the very fine resolution of 30 arc-seconds but is a slightly older model built under CMIP5 (Taylor et al. 2012), whereas the WorldClim dataset uses ACCESS-ESM1-5 at a larger resolution of 2.5 arc-minutes but was built under the newer CMIP6 protocol (Eyring et al. 2016). Most importantly, the different datasets were built under different climatological standardization guidelines, so while WorldClim uses Shared Socioeconomic Pathways (SSPs), CHELSA uses older Representative Concentration Pathways (RCPs). This presents a subtle difference, as SSPs are calculated with the consideration of factors such as technological development, population, and governance, alongside greenhouse gasses while RCPs simply base calculations on atmospheric greenhouse gas levels to determine radiative forcing (Meinshausen et al. 2020). Despite this series of small differences, we saw largely concurrent values among our base (current) climate for each sampling area with pairs.panels tests from the R package 'psych' v.2.4.6.26 (Revelle 2024) of both datasets showing similar signals of collinearity among terms (detailed in next section). While the 19 bioclimatic variables from CHELSA and WorldClim showed similar patterns of collinearity for the current climate (Figures S7, S8), we sought to investigate whether subtle differences in their underlying construction and downscaling algorithms would propagate into divergent predictions of future climate vulnerability.

Gene environment associations and local adaptation

In order to identify genetic variants associated with the climate we used the shared 19 bioclimatic variables from both the CHELSA and WorldClim climate databases and incorporated topographic data from GeoNames to explore if some populations might have adaptation for high or low elevations as an additional explanatory environmental variable. Two individuals (NL1003 and NL1007) from Newfoundland had coordinates located on water on the eastern border between Canada and the French Overseas Territory of Saint Pierre and Miquelon and accordingly had their position adjusted approximately 15km east southeast to 46.9892019, -55.941208 so that bioclimatic data could be read in. These changes were retained for all subsequent testing. Additionally, R packages 'geonames' (Rowlingson 2019) and 'elevatr' (Hollister 2023) could not pull data for several remote high latitude areas so individuals 9:28, 41:49, and 69:99 had the data manually retrieved using data held in Leaflet (Agafonkin et al. 2010). With complete environmental terms available for each sampling site we performed a pairs.panels assessment using the 'psych' package in R to determine which climate data showed high collinearity. We used a standard correlation threshold of ±0.7 where anything larger was discarded, leaving us with 7 independent environmental variables for assessment. Two exceptions to the cutoff were made, we retained Precipitation (PRE) in the WorldClim set (r = .72 with Annual Mean Temperature [AMT]) and Elevation (ELE) in the CHELSA dataset (r = 0.85 with Mean Diurnal Range [MDR]). Additionally, CHELSA future climate data required rescaling for AMT, MDR, Seasonality (SEA), and Mean Temperature of the Wettest Quarter (MWE) by a factor of 0.1 to ensure consistency with past climatologies. After final environmental variables were chosen, we checked the effect of latitude and longitude and observed moderate collinearity between latitude (LAT) and AMT for both the CHELSA and WorldClim datasets (-0.75 and -0.84 respectively; Figure S7), but also between LAT and PRE for WorldClim (-0.78; Figure S8). These relationships are expected and the trends are intractable from large scale climate data considering that temperature invariably drops and precipitation often decreases at increasing latitudes. We address below that this could have some impact on interpretation of downstream analysis by inflating correlations of higher precipitation and warmer temperatures in the more southerly populations. A summary table of the environmental variables used in the final analysis and pair correlations are provided in Table S2.

We used two complimentary methods to identify gene-environment associations for all individuals together as well as for each species separately. First, we used latent factor mixed models (LFMM) implemented in the LEA package v.3.12.2 (Frichot & Francois 2015) and second, we used the standard co-variate model in BayPass v.2.41 (Gautier 2015, Olazcuaga et al. 2020). By using two complementary methods a more robust inference is potentially possible. If both methods, despite their different statistical foundations, identify similar patterns or genes, the confidence in those findings is increased. Subtle differences between these association methods are described below.

LFMM estimates the effect size of environmental variables on allele frequencies while estimating and correcting for demographic history and other neutral processes partitioned into K number of unobserved latent factors. We used the lfmm2 function in LEA , and as suggested by the snmf test we set K to 10 for the full VCF, and K set to 6 and 4 for *L. muta* and *L. lagopus/scotica* respectively. This produced a set of p-values correlating frequencies of each variant to each of the environmental parameters. We performed a

p value adjustment to account for multiple testing and reduce false discovery rates with the R package 'qvalue' (Storey et al. 2025) to produce an adjusted 'q value' associated with each variant test. The BayPass core model is free of co-variates and estimates the population covariance matrix describing the demographic history of the populations in questions. The BayPass standard co-variate model extends the core model by estimating linear association between genetic variants and environmental co-variates while using the population covariance matrix for correction of neutral processes. We created the input files for BayPass by first converting the VCF files into Treemix format using The Popgen Pipeline Platform (PPP; Web et al. 2021), and then used basic bash scripting to get the treemix file into appropriate BayPass format. We then sub-sampled the data into individual data sets consisting of 100,000 SNPs and according the recommendations in the BayPass manual for large data sets, using poolfstat v.3.0.0 (Hivert et al. 2018, Gautier et al. 2022, Gautier et al. 2024). We ran the core model in BayPass v.2.41 (Gautier 2015, Olazcuaga et al. 2020), on each of the pseudo-independent data sets (full VCF, *L. muta* only, and *L. lagopus/scotica* only ; labeled as ptarm, muta, and lago respectively) to estimate a population covariance matrix to account for population structure and demographic history, to be used when estimating environment associations. We ran 3 independent runs with a different random seed for each data set. We used the inferred population covariance matrix as input for the standard co-variate model and used the importance sampling (IS) approximation to estimate Bayes factor for each association. As for the core model, we ran each data-set for three independent runs to account for between run variance in the estimation of Bayes factor. We used the median Bayes factor from the three independent runs and a cutoff of BF > 10 to consider it as evidence of a SNP-environment association.

Gene ontology analyses

The position of the variants significantly associated with environmental variables or identified as differentiation outliers from BayPass and LFMM, was matched to the rock ptarmigan annotation file (Squires et al. 2023) to extract the gene names using BEDtools (Quinlan & Hall 2010), considering only SNPs that intersect with an annotated gene. These were used for input to g:Profiler (Kolberg et al. 2023), searching against chicken gene ontology terms using the g:SCS threshold to correct for multiple testing and a significance cutoff 0.01. In addition to running manual intersects of SNP positions and gene names we used the R package 'Venn.Diagram' to visually compare overlap between terms for each model and data set.

Genomic offset under future climate change scenarios

In order to assess rock and willow ptarmigan vulnerability to future climate change we estimated the genomic offset between allele frequency associated with the current environment and associations with the environment projected under two different climate change states for each model. For CHELSA this was RCP 4.5 and 8.5, and for WorldClim it was ssp 245 and 585. These predicted climate states are relatively similar, with RCP 4.5 approximately equivalent to ssp 245 (Most likely climate scenario; hereafter combined as RCP 4.5), and RCP 8.5 approximately equivalent to ssp 585 (Worst case scenario; hereafter combined as RCP 8.5). For each sampling location we checked the same environmental variables that were used in our analysis and included environmental projections from the two RCP trajectories for the window 2060-2080. Elevation could not be included in the offset calculations as it is not typically handled in climatic datasets and is not changing as rapidly as the climate environment.

To estimate genomic offset we employed three different strategies. First, we estimated offset for all SNPs using the genetic_offset function in LEA. Second, we used the built-in function in BayPass directly comparable to the offset method in LEA on the estimated beta coefficients for all SNPs. And finally, we used the candidate SNP approach in LEA, estimating genetic offset using SNPs identified by lfmm after multiple testing adjustment (q<0.05) and Baypass (BF > 10) in separate runs. The BayPass cutoff at BF10 is recognized as strong and robust support for correlations (Lee and Wangenmakers 2014).

We interpret offset as the adaptive miss-match between a population's current allele frequencies associated with climate variation and and a hypothetical version of that population with allele frequencies associated with a projected future climate, where the geometric offset statistic has a similar interpretation as D_{ST} (Gain et al. 2023). This method assumes weak and polygenic effects of the environment spread throughout the genome.

Results

Population structure and Diversity estimates

Rock ptarmigan populations exhibit lower genomic diversity and stronger differentiation than willow ptarmigan/red grouse. Our generated sequence data had an average depth of coverage of 34.2X and on average 98.8% of the reads aligned to the reference genome (Table S3). L. *muta* autosomal π ranges from $9.3*10^{-4}$ to $3.2*10^{-3}$, among the different populations while the lowest π can be found in the birds from Svalbard ($\pi = 9.3*10^{-4}$), the French Pyrenees ($\pi = 1.5*10^{-3}$), and Iceland ($\pi = 1.7*10^{-3}$; Figure S9). For *L. lagopus/scotica* populations we found autosomal π ranges between 3.6*10 -3 and 4.3*10 -3 (Figure S9). Comparisons of Weir-Cockerham F_{ST} values show that f or *L. muta* observed F_{ST} values ranges between 0.0332 and 0.629 with birds from the isolated Svalbard and the Pyrenees appearing to be the most differentiated populations generally (Figure S10). Among *L. lagopus/scotica* F_{ST} ranged between 0.018 and 0.288 with birds from Newfoundland showing the largest differentiation and relatively close values between birds in Scandinavia and the British Isles (Figure S10). Within *L. muta*, populations showed generally lower Dxy values than *L. lagopus*. Among *L. muta* we observed Dxy between 0.0043 and 0.0057 (Figure S11) while *L. lagopus/scotica* showed values from 0.0076 to 0.0061 (Figure S11).

Over-interpretation of K values can lead to misleading conclusions about population structure and admixture events, as it often assumes that the highest supported K value output by algorithms represents the true number of ancestral populations, which may not always be the case (Janes et al. 2017; Meirmans 2015). We had several options for assigning K values in tests to estimate the number of analyzed populations. According to ADMIXTURE analysis of the whole dataset, K=5 (cv.error: 0.4038) showed the best fit. However the cross-validation error was relatively flat and similar between K=3 and K=11(Avg: 0.4202; SD: 0.0154)(Figure 3).

This implied validity of K=10 (cv.error: 0.4323) for the whole dataset, as the ADMIXTURE analysis for all groups was not completely ideal with K =8 and 9 suggesting equally good model fits (Figure S5). K10 lumps West and East Greenland as a single group, but *L. muta* from Sweden and the Alps also remain

joined. At K10 some separate grouping in the Norwegian/Swedish population was observed in *L. lagopus* while other groups remained well resolved.

LEA provides an ancestry matrix based on the Q scores from snmf. Assuming K=10, it almost exactly matches the K=10 result from ADMIXTURE with the exception that *L. muta* from Sweden and the Alps were split, while British and Irish *L. scotica* were separated and within regional structure appearing only in Norwegian *L. lagopus*. Taking all of these results into consideration, we settled on using K = 10 in down-stream analysis of the full dataset, K = 6 for tests of *L. muta* only, and K=4 for combined tests of closely related *L. lagopus/scotica*.

Gene environment associations and local adaptation

Both climate datasets identified a large number of climate-associated SNPs, though CHELSA consistently identified more candidates than WorldClim across all analyses (Table S4, S5). A key finding was the limited overlap in the specific SNP loci identified by the two climate datasets (Figure 3), even when using the same association model. Seven non-collinear climate terms were deduced through pairs.panels assessment and tested for association with SNPs with six of these being from standard outputs for bioclimatic variables and the seventh being elevation (Figure S7 and S8). The final variables used were Annual Mean Temperature (AMT), Mean Diurnal Temperature Range (MDR), Seasonality (SEA), Mean Temperature of the Wettest Quarter (MWE), Precipitation (PRE), Variability of Precipitation (PVA), and Elevation (ELE).

In total we identified 6,268,635 biallelic SNPs among all study individuals in the final LD pruned VCF. Examining species groups separately meant that we had new invariant sites specific to *L. lagopus/scotica* and *L. muta*. Accordingly, within these separated groups we found *L. lagopus/*scotica had 4,973,265 SNPs and *L. muta* had 4,702,905 SNPs respectively. Even though more SNPs were available for comparison with the whole dataset comparisons including all individuals, a larger number of SNPs significantly associated with environmental variables ($q < 0.05$) were captured by using the separated species groups.

In order to confirm that our initial BayPass runs converged we measured the correlation between runs of the core model for the population covariance matrix (full vcf: r^2=0.986-1.0, muta: r^2=0.999- 1.0, lago: r^2=0.997-1.0). We also used the R function fmd.distance() supplied with BayPass to assess convergence of the population covariance matrix between runs (full vcf: 0.0041 – 1.0447, muta: 0.0010 – 0.00276, lago: 0.0013 – 0.3844). The correlation statistic close to one and the small fmd values indicate that the runs of the core model converged.

For the full VCF with all individuals included, BayPass identified 139,492 unique significantly associated SNPS and 10,913 genes, while LFMM identified 92,585 unique significantly associated SNPS and 9923 genes. While less than 7% of predicted SNPs were shared between climate datasets under LFMM, 32% were shared under BayPass. Additionally we observed that over 85% of all predicted associated genes were shared between the models and associated genes represented almost half of all annotated genes known for the Rock Ptarmigan (Figure 3).

For the LEA model with the CHELSA dataset we observed strong associations of 54438 unique SNPs with environmental variables ($q < 0.05$). With tests on both species 21.7% of significant SNPs under the CHELSA dataset showed significant association with two or more environmental terms. These included hits on AMT: 5684; MDR: 18188; SEA: 7605; MWE: 12564; PRE: 8787; PVA: 7913; and ELE: 9936. With the WorldClim dataset we observed strong associations of 37781 unique SNPs with environmental variables ($q < 0.05$). With tests on both species 12.5% of significant SNPs under the WorldClim dataset showed significant association with two or more environmental terms. These included hits on AMT: 4609; MDR: 2427; SEA: 12130; MWE: 5606; PRE: 9943; PVA: 4429; and ELE: 5871.

We observe that CHELSA consistently produces more strong associations than WorldClim in all test conditions. When species groups were broken down, we saw that CHELSA produced 135486 unique associated SNPs for *L. lagopus/scotica* and 163411 for *L. muta* while WorldClim found 78799 unique associated SNPs for *L. lagopus/scotica* and 96460 for *L. muta*. This also shows a larger number of significant hits when testing across *L. muta* exclusively than when testing on L. lagopus/scotica SNPs or variants from both groups combined. For the BayPass model under CHELSA we observed 115,958 strong associations of SNPs with environmental variables ($p < 0.001$). These were AMT: 23274; MDR: 17071; SEA: 8540; MWE: 23954; PRE: 10377; PVA: 8015; and ELE: 24727. Using the WorldClim dataset, BayPass found 104,706 strong associations. Those were AMT: 23366; MDR: 6697; SEA: 6430; MWE: 16870; PRE: 18140; PVA: 8544; and ELE: 24659.

For both LFMM and BayPass, association tests were also run on subset populations and all results are summarized in Table S4 and S5. We found many ontology terms significantly enriched with genes containing the SNPs identified by our gene-environment analyses (Table S6a-d). In short GO-terms under "Biological Processes" were regularly associated with neurogenesis (and other terms linked to neural function) for all test conditions. We also found the most significantly enriched GO-terms were often for general development such as regulation processes and cellular organization. While the specific SNPs associated with climate showed little overlap between analyses, the genes containing these SNPs were highly concordant (Figure 3). Across all models and datasets, GO enrichment analyses consistently pointed to terms associated with 'neurogenesis' and 'general development', suggesting that adaptation to climate involves a conserved set of physiological and behavioral pathways, even if the specific mutations differ among populations.

Offset under future climate change scenarios

Expected offset was used to predict the risk for each study population across multiple climate scenarios. Clear signals of higher genomic offset were found for Svalbard and Greenland for *L. muta* while *L. lagopus/scotica* showed highest predicted values in Ireland, Newfoundland, and Newfoundland (Figure 4). We found clear differences between populations when running LEA's genomic offset tests on all individuals together at $K = 10$ (Table 1). *L. muta* in the Alps produced the largest individual genomic offset values with the RCP 8.5 CHELSA climate dataset for both BayPass and LEA, though there was high variance within the population. While these values should not be considered an extreme vulnerability alone, they are high compared to the other populations and difficult to contrast with other systems given that offset tests are individual and not directly comparable. We observed that these numbers changed depending on how many populations we ran the analysis with, and that lower K values

produced rapidly increasing offset estimates. This trend may have explained why later tests within species groups with smaller K values and subset sample sizes produced significantly larger offset values. For the main test of LEA however, the smallest observed value was in a *L. lagopus* from Sweden from a RCP 4.5 run with climatic data from CHELSA. An *L. muta* from Iceland, produced a similar value under these arrangements. Under BayPass, the lowest observed value was the same Icelandic bird. BayPass in general produced higher offset scores with more variance (see Figure 4). Although the different calculation methods between BayPass and LEA make the exact results not directly comparable, we observe similar trends overall (Figure 4; detailed further in S12 through S15 and Table S7 and S8).

Change in offset values between future climate models seemed to be slightly larger for those calculated under CHELSA, so that the more severe climate conditions resulted in a higher impact on offset compared to moderate climate change than under WorldClim predictions (Figure 4). In only one instance, LEA's calculations among the birds of west Greenland under the CHELSA dataset, did we observe that the more severe climate change predictions actually improved local offset risks though the actually decrease in risk was quite small (Average offset reduced -0.004; Figure 4).

The offset was also calculated for sets of each species group separately but these tests seemed to strongly inflate some of the scores (calculated values over 1.0 suggesting extreme offset in *L. lagopus* of Norway and Newfoundland were probably exaggerated) leading us to have to lower confidence in the results from these tests. This likely occurred due to the smaller number of individuals in each group informing the environmental correlation, however despite some large outliers, the general trends closely matched with our original analysis of all individuals together. The candidate SNP approach was conducted on a reduced set of SNPs for each test arrangement and appeared to produce a similar phenomena with the final offset values loosely reflecting the full SNP results for both climate datasets and total offset calculations greatly inflated (Figure S16 and Table S9). For WorldClim data under the BayPass model this included 104,706 SNPs, while BayPass under CHELSA data included 115,958 SNPs. For WorldClim data under the LEA model this included 37,781 SNPs while CHELSA under the LEA model used 54,438 SNPs. Notably the candidate SNP approach appeared to generate greater disagreement between the different association models (Figure S16). Among other trends these alternative tests agreed that birds from Norway and the French Alps had elevated offset, while *L. muta* from Sweden and Iceland appeared resilient to expected environmental change.

Discussion

Understanding how species respond to environmental change is crucial in the context of rapid climate shifts, particularly for organisms inhabiting extreme environments. Successful adaptation for any population necessitates that standing genetic variation is available and/or phenotypic plasticity is present. Enough time must also be available for natural selection to act upon the available diversity. Rapid environmental change may limit the ability for local populations to track these changes (Hoffmann & Sgrò 2011). Even though the ptarmigan populations in our study have been geographically isolated for thousands of years, they provide a good model for studying genomic diversity, adaptation, and vulnerability to climate change due to their widespread distribution across the North Atlantic and Arctic

regions. In this study we identified differences in local adaptation among geographically separated populations as well as evidence of varying genomic offset to future climate change. The three main results of our study are: (1) patterns of genomic diversity are shaped by both natural selection and drift in geographically isolated populations; (2) climate adaptation is highly polygenic, with evidence for convergent evolution at the gene-function level; and (3) predictions of climate vulnerability are highly contingent on the choice of underlying climate models and association methods, leading to conflicting conclusions of importance to conservation.

Genomic diversity

The species in this study have either truly circumpolar (rock ptarmigan) or near circumpolar (willow ptarmigan/red grouse) distributions (Storch 2000). Though all three species are generally sedentary, closely co-occurring populations are connected by gene flow and more isolated populations have been separated since the last major glacial period creating patterns of isolation by distance (Sahlman et al. 2009, Höglund et al. 2013). This explains why Newfoundland willow ptarmigan (*L. lagopus alleni*) are more genetically distant from Norwegian willow ptarmigan (*L. lagopus lagopus*) than red grouse (*L. scotica)*. In a similar fashion, the differentiation between *L. muta* populations in Svalbard and the Pyrenees comes from at least tens of thousands of years of separation. For several populations around the North Atlantic, the ocean has acted as an effective barrier to gene flow. In the case of rock ptarmigan, the North Atlantic Ocean has been a less obvious barrier as this species is adapted to even more extreme arctic climates. The rock ptarmigan has successfully colonized Greenland, Iceland, and Svalbard after the most recent glacial periods, areas from which the willow ptarmigan/red grouse are absent today. Previous studies have suggested that Svalbard was colonized by rock ptarmigan from the east (Taymyr Peninsula), while the origin of birds colonizing Iceland is less known (Sahlman et al. 2009). We observed lower genomic diversity among all *L. muta* populations than in any of the *L. lagopus/scotica* populations with the exception of rock ptarmigan from the Alps and red grouse from Ireland. In agreement with previous research, we observed the lowest nucleotide diversity among birds in Svalbard, the Pyrenees, and Iceland. Compared to other bird species the diversity among ptarmigan appears moderately high (Dutoit et al. 2017; Backström et al. 2008; Lamichaney et al. 2015). High diversity was previously reported for Scandinavian *L. lagopus* (Berlin et al. 2008).

Environmental Associations and Local Adaptation

We used BayPass (Gautier 2015) and LFMM in LEA (Frichot & Francois, 2015), as they offer a way to correct for the underlying population structure caused by the geographical isolation among our populations (Olazcuaga et al. 2020). We found many SNPs associated with climate and relatively little overlap when comparing SNPs between species. We see that different polymorphic sites become significantly associated when individual population groups are separated out from tests with all individuals. We also observe a general trend that CHELSA climatologies produced more significantly associated SNPs than WorldClim. The environmental terms with the most highly associated SNPs varied largely by test condition. In general, 'Mean Temperature of the Wettest Quarter' and 'Precipitation' had many hits in all tests but 'Annual Mean Temperature', 'Variability of Precipitaion', and 'Elevation' never scored in the top two strongest associations for any test. Overall, this indicates that the source of environmental data used in association can seriously alter conclusions regarding drivers of adaptive

variants. Furthermore, the relatively high proportion of variants that are significantly correlated to multiple environmental terms could suggest pleiotropic effects where environmental terms have low collinearity.

When investigating SNPs within protein coding genes the number of hits was reduced and the overlap among species was much greater. This suggests two things. First, "climate adaptation" is polygenic and involves a larger number of SNPs throughout the studied genomes. Second, the greater overlap suggests changes may occur and affect similar physiological pathways. This in its turn may support that there are multiple ways for populations to adapt to changing climates but a limited number of genomic regions that may be the target of selection. For example, past research has shown clear relationships between snowfall and reproductive success among Rock Ptarmigan populations in the Alps and the Pyrenees (Imperio et al. 2013, Novoa et al. 2008, García-González et al. 2016). Rainfall has been observed to negatively influence chick survival in Icelandic rock ptarmigan (Ólafur Karl Nielsen *pers. comm.)* while warm and dry conditions have been shown as an important factor in brood success for the related white-tailed ptarmigan (Wann et al. 2016). This relationship with precipitation is a widely recognized trend among grouse often intertwined with temperature (Wegge et al. 2022).

We must note some observed collinearity between the sampling latitude of our sequenced individuals and the environmental terms of annual mean temperature and precipitation (Figure S7 and S8). This was unsurprising as both temperature and precipitation are widely recognized to decrease near the poles, and a significant portion of our samples were taken at high latitude. However, some of the increased genetic risk calculated at high latitude may be inflated by this relationship. The arctic is expected to warm up to four times faster than the rest of the planet (Rantanen et al. 2022). Although the currently established genetic relationships with these variables might be over-represented by distance effects, we also must recognize that the environmental shift expected in these areas is likely to be larger than at lower latitudes.

Given some ptarmigan groups' stronger correlation with elevation we suggest that rock ptarmigan populations in the Pyrenees and the Alps could be more genetically tailored to high elevation and local habitats than to the specific climatic conditions on mountains. This could encouragingly imply that these birds are less specialized for cold niches and will be capable of surviving on warmer mountain tops with less snow, or alternatively suggests that ptarmigan distribution is strictly habitat dependent and that erasure of cold-adapted plant species and encroaching tree coverage could pose the most immediate problems. Such risks are addressed thoroughly in recent works (Chamberlain et al. 2023).

Local adaptation and gene enrichment

We found a low SNP overlap but high gene overlap among comparisons using different data sets and methods. This pattern strongly suggests that adaptation is not occurring via a few major-effect genes but is highly polygenic, involving many loci of small effect. The fact that different SNPs are implicated in the same genes across different populations or species suggests convergent evolution at the functional level. Different populations have arrived at similar adaptive solutions (e.g., modifying neural or developmental pathways) via different genetic mutations. This pattern strongly suggests that adaptation is not occurring via a few major-effect genes but is highly polygenic, involving many loci of small effect.

The most often found GO-term under "Biological Processes" using both CHELSA and World Clim data for both species combined and separately were associated with neurogenesis and general development. Our results also suggest that there are multiple ways to respond to intricate environmental changes associated with climate. These responses may involve both behavioral and developmental changes and differ among populations.

When SNP associations under each climatology (CHELSA or WorldClim) are compared, there is a limited amount of overlap in the sites shared between the different climatological models (See figure 3). These trends held when individual species groups were tested. This suggests that there are important differences in how the different climate models determine scores for local climatological variables, how these are associated with genetics, and therefore how adaptive variation can accurately be identified.

When looking at which tested non-collinear environmental variables were producing the most significantly associated SNPs, Annual Mean Temperature, Mean Temperature of the Wettest Quarter, Precipitation, and Elevation were the most important variables according to BayPass (Table S4). CHELSA produced a stronger association with Mean Temperature of the Wettest Quarter than WorldClim (most important, vs. fourth most important). Under the LEA model, CHELSA data produced more associated SNPs for the metric of Mean Diurnal Range, and Mean Temperature of the Wettest Quarter. Though in general there were many SNPs identified with each variable. For LEA's WorldClim run on the other hand, seasonality and precipitation appeared most important though again all terms showed associations (Table S5). This suggests that between assessment models and climate data there was no clear best term and thus specific environmental factors may act in concert to drive selection.

By comparing the associations produced by a LFMM from LEA and a Bayesian standard covariate model from BayPass we observe high overlap in genes with a predicted association to environmental variables. The concurrence of these two methods suggests that the associations are reliable. Roughly half of all annotated genes for Ptarmigan showed SNPs with significant association to environmental factors across all test orientations. This is unsurprising as environment is heavily responsible for survival and life history, and the functional associations of genes to the environment are likely holistic and multigenic. When we compare only the association of SNP positions between groups, we see a much lower amount of overlap suggesting that these incongruent SNPs are actually located in the same genes across population groups and species. Different polymorphisms are therefor likely acting on the same genes, potentially in the same way between groups. It bears reminding however that the same genes can easily be re-appropriated to perform new functions (Jones et al. 2012; Mack et al. 2018). In the future, a detailed examination of expression patterns may help to resolve if up-regulation or down-regulation of associated sites is consistent in responses to environmental change.

Genomic offset under future climate change scenarios

Our main genomic offset results found high vulnerability in the Alps, Svalbard, and Greenland. These results rest on the assumptions of the models used to calculate genomic offset: i.e., populations are assumed to be found in an adaptive equilibrium, and space-for-time substitutions may be used to infer the outcomes of a warmer climate (Rellstab et al. 2021). These predictions, however, must be interpreted with caution. Our finding that elevation—a static environmental factor—is a strong selective pressure for

some populations suggests this assumption may be only partially met, potentially confounding climate-based predictions. A high offset value does not necessarily equate to a simple, linear decline in fitness and may not capture a population's full adaptive capacity (Ahrens et al. 2023, Gain et al. 2023). We also observe that inferred population structure may influence offset predictions with higher K values consistently producing lower offset estimates for our genetic data.

A further contention is that the choice of climate dataset led to different populations being identified as most vulnerable. This discrepancy is not random; it reflects fundamental differences in how the models are constructed. CHELSA incorporates algorithms designed to better represent orographic effects, which may explain the higher offset values predicted for topographically complex regions like the Alps. In contrast, WorldClim's more recent versions utilize different data sources that may alter predictions in other regions. Vulnerability assessments must explicitly account for this source of uncertainty, perhaps by using an ensemble of climate datasets in the same way that ensembles of General Circulation Models (GCMs) are used (Steen et al. 2017).

Genomic diversity and demographic history in the context of ancient and future climate models to infer long-term range shifts have previously been studied in both willow ptarmigan and rock ptarmigan (Lagerholm et al. 2017; Kozma et al. 2018). For both species, available habitat was predicted to be reduced under relevant future climate scenarios and hence the range area to become smaller by about than 40%. The less cold-adapted willow ptarmigan range is predicted to shift towards higher latitudes and altitudes thus enabling some compensation for habitat loss due to warmer climates, while the range of the more cold adapted rock ptarmigan will be increasingly limited under these conditions (Kozma et al. 2018.) Hence, rock ptarmigan cannot move as readily and will be forced to adapt to future climatic conditions within the current range. Previous studies integrating population genomics and environmental data have identified genomic variation associated with changing climate conditions across the breeding range of a migratory songbird, the yellow warbler (*Setophaga petechial*, Bay et al. 2018). Warbler populations requiring the greatest shifts in allele frequencies to keep pace with future climate change had experienced the largest population declines, suggesting that failure to adapt may have negatively affected populations. In the arctic, maladaptation has been predicted to be widespread among polar bears in the Canadian north, with some subpopulations promising higher adaptability to changing sea ice conditions than others (Rivkin et al. 2024).

As alluded to in the introduction, high offset indicates a significant adaptive challenge without necessarily providing a clear or realistic estimate of a population’s adaptive potential. Many other factors such as new immigration, behavioral plasticity, and changed competition or predation may influence population success beyond the immediate genetic standing and expected climatic changes. While the offset for ptarmigan in this study did not always agree between models, we expect this is an artifact of poor resolution of some climatologies at high altitude and latitude. Models agreed that Icelandic and Swedish *L. muta* showed low offset, while Swedish *L. lagopus* also had low scores across models. Compared to the other species groups, *L. scotica* of Britain and Ireland seemed to show the lowest offset. Both CHELSA and WorldClim datasets also suggested that *L. lagopus* from Norway had a higher offset. Other populations showing potentially high genomic offset in our analysis included *L. muta*

from Svalbard and the Alps according to CHELSA's predictions and both populations from Greenland according to the WorldClim datasets.

Calculating offset using candidate SNPs seemed to significantly increase the incongruence between association models and generally elevate the scale of calculations (compare Figure 4 with Figure S16) while largely mirroring the trends seen with the full set of variants. To this end it is unclear the extent to which offset predictions based on candidate SNP approaches are actually more informative or reliable when compared to the full model including all variants as associations are heavily dependent on the reliability of environmental data.

Overall the findings suggest genomic offset was elevated under future climate scenarios built under the assumption of increased greenhouse gas emissions (RCP 8.5, SSP585) for both the CHELSA and WorldClim datasets. While WorldClim produced higher offset predictions on average, CHELSA had greater stochasticity and variance producing larger differences between the tested concentration pathways. This was also true of BayPass offset results when compared to those from LEA We observed that *L. muta* from the east of Greenland had comparatively high offset values under the WorldClim data, while *L. muta* from Svalbard showed heightened risks under the CHELSA data. This discrepancy between datasets likely arises due to poor resolution of data in remote areas and at higher latitudes with fewer historical weather stations in the Arctic. Both models agreed that populations of *L. lagopus* from the interior of Norway showed high offset under more severe climate scenarios. Willow ptarmigan from England and Ireland (red grouse) had lower offsets than other populations. Many of these trends were intensified with the candidate SNP approach to offset calculation (compare Figure 4 with Figure S16).

For rock ptarmigan, genomic diversity alone suggests that birds from Iceland, Svalbard, and the Pyrenees are at higher risk from climatic shifts while populations in Scandinavia and the Alps are projected to be the least affected. The populations of red grouse on Great Britain and Ireland show the lowest levels of diversity among the *Lagopus lagopus/scotica* group (though still higher than most rock ptarmigan and many other bird species). Despite this, a relatively stable future environment in Great Britain and Ireland shows that genomic offset is not as pronounced as for willow ptarmigan in Newfoundland which are expecting larger changes in average temperature and rainfall. Similarly, despite poor genomic diversity and high latitude, genomic offset scores suggest that Icelandic rock ptarmigan may in fact be less threatened than expected based solely upon climatic associations of the population.

The fact that the more northern populations are generally predicted to be more vulnerable to future climatic change is line with observations of Arctic ecosystems being the most immediately impacted by climate change (Post et al. 2009; Box et al. 2019). A study of two cryptic European forests bats concluded that by considering climate-adaptive potential, range loss predictions were reduced as compared to scenarios which did not allow for adaptive evolution (Razgour et al. 2019). For *L. muta,* elevated offset (and thus vulnerability) is predicted particularly around Greenland and Svalbard which concurs with more severe climatic shifts anticipated in the high arctic. The French Alps also showed elevated values. For *L. lagopus/scotica* high genomic offset was projected in Newfoundland, and some vulnerability in Scandinavia was retained while there appeared to be much lower levels of offset in Great Britain and Ireland.

Conclusion

In conclusion, our comprehensive genomic analysis of North Atlantic ptarmigan reveals that while local adaptation to climate is pervasive and highly polygenic, our ability to predict future vulnerability is fundamentally constrained by uncertainty in the data used for forecasting. We show that different state-of-the-art climate datasets yield conflicting assessments for at-risk populations, highlighting a critical challenge for conservation genomics: the accuracy of our sophisticated genetic predictions is ultimately limited by the accuracy of the environmental data upon which they are built. Similarly, accurate understanding of population structure may have a bearing on conclusions as increasing population breaks appear to decrease calculated offset values. This study underscores that disentangling the effects of neutral demographic history from selection remains a key challenge (Storfer et al. 2018). We advocate for an approach to conservation that integrates multiple sources of uncertainty and focuses on preserving the functional genetic diversity that underpins the complex, and likely redundant, adaptive responses of species to a changing world.

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Statement on Conflicts of Interest

We the authors declare that we have no identifiable conflicting or competing interests relevant to the contents of this manuscript.

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Figures

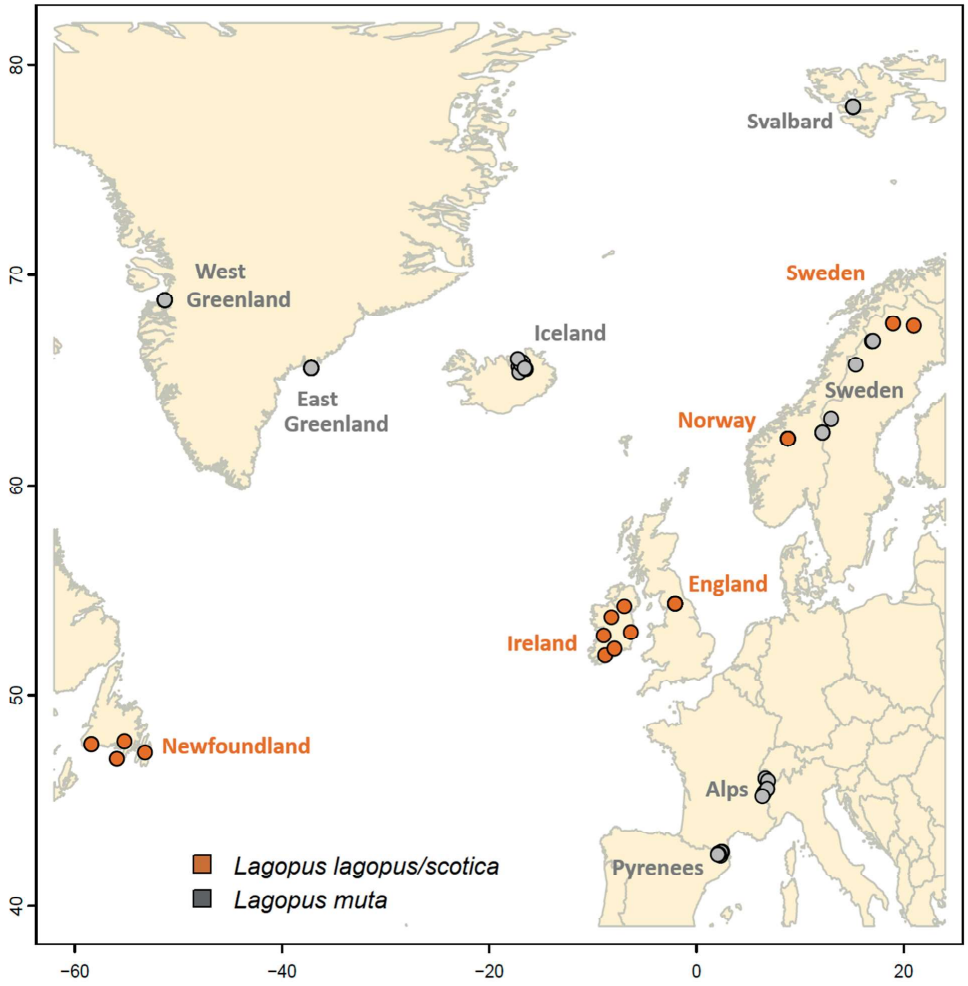
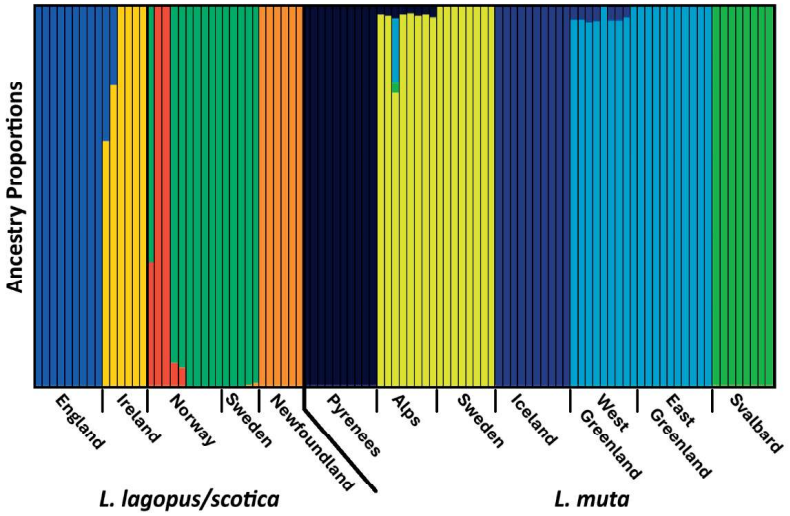


Figure 1. Distribution of our sampled populations around the North Atlantic and Arctic with different *L. lagopus/scotica* sampling points colored in brown and *L. muta* in gray. Note that there are some populations where sampling points overlap completely, and others where they are partially obscured due to nearby placement of several individuals. A complete list of sampling coordinates and site information is available in Table S1.

a)



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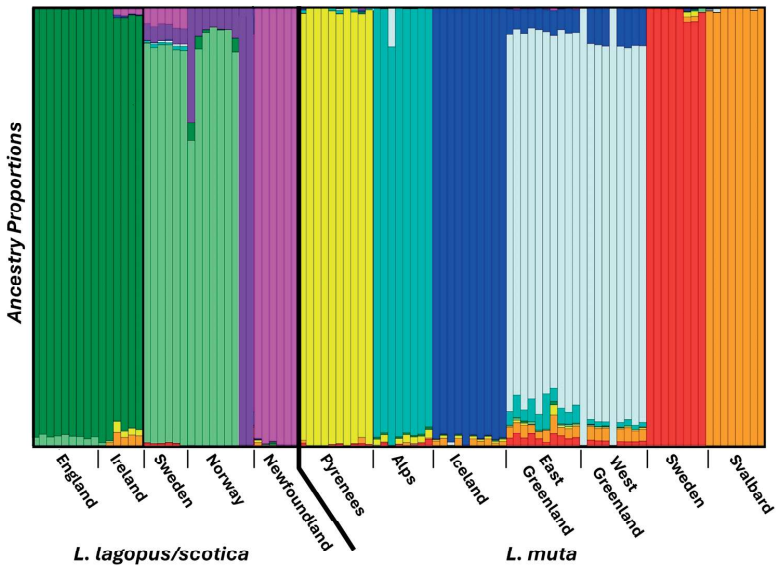

Figure 2. a). Admixture plot showing population breakdown at K=10 produced by pulling Q values into the Rshiny app 'Pophelper'. b) Ancestry matrix at *K* = 10 produced by LEA which was calculated as the optimal arrangement of individual ancestries in the dataset. Note that some positions are changed between plots.

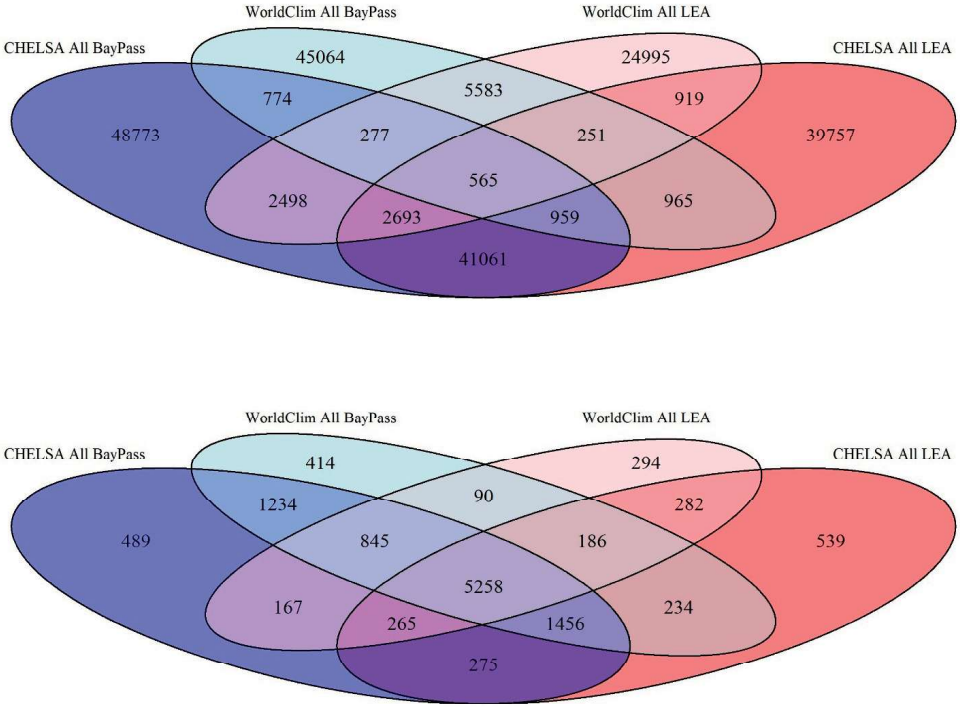

Figure 3. Venn diagram developed from the R package 'venn.diagram' and data from both 'LEA' (in red shades) and 'BayPass' (in blue shades) showing overlap in the number of unique SNPs (above) and genes (below) very significantly associated with environmental parameters after adjustment for multiple testing ($q < 0.05$) according to a Latent Factor Mixed Model regression analysis and Bayesian standard covariate model on both WorldClim and CHELSA climatologies and elevation data for all individuals (at K=10). Significant overlap is shown in genes between all models with 43.7% shared among all arrangements of climate model and association tests, while SNPs are shared between all models and climate sets in less than 0.3% of cases.

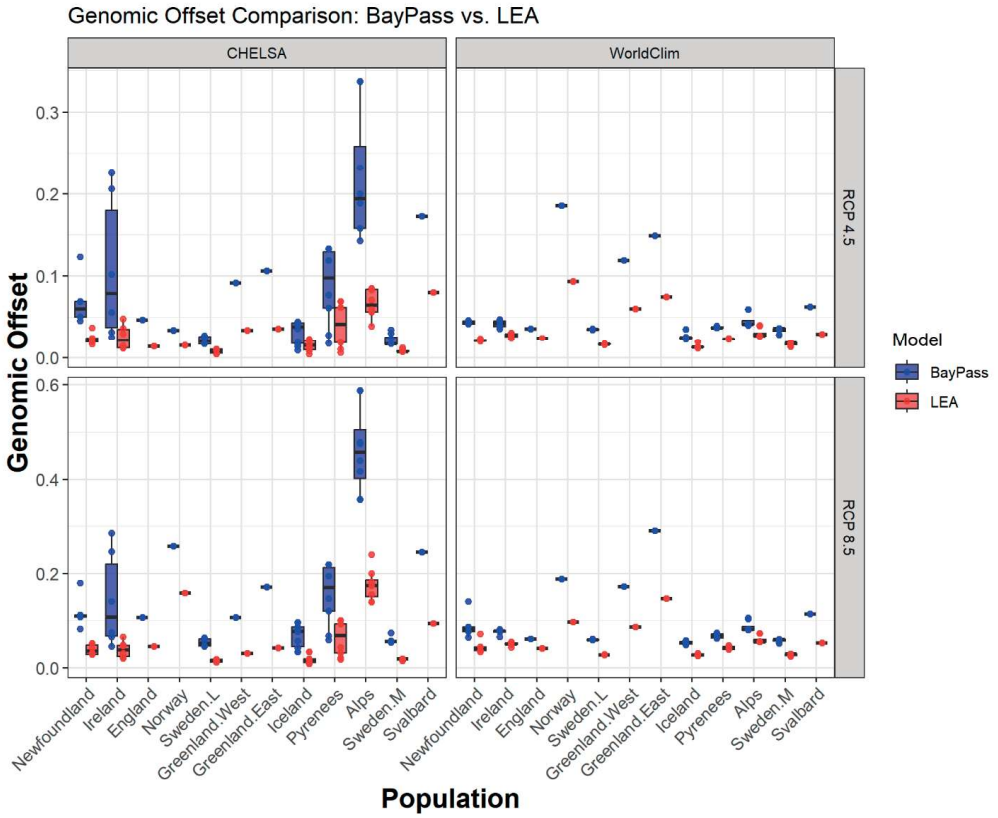

Figure 4. Figure showing four grouped box plots indicating the genomic offset scores for each of the study populations under various scenarios as calculated by BayPass (Blue) and LEA (Red) using the full variant set of 6,268,635 SNPs. These include the different climate datasets from CHELSA and WorldClim, along with the different relative concentration pathways tested at RCP 4.5 and RCP 8.5. Each plot is split with a line separating the *L. lagopus/scotica* group on the left, and the *L. muta* group on the right. Note that the Y axis for offset is scaled differently under the different concentration pathways. Average values for each population are outlined in Table 1 while scores for individuals under LEA and BayPass can be found in Table S7 and S8.

	LEA				BayPass			
	CH45	**WC45**	**CH85**	**WC85**	**CH45**	**WC45**	**CH85**	**WC85**
L. lagopus/scotica	0.0171	0.0367	0.0589	0.0518	0.0555	0.0684	0.1355	0.0948
Newfoundland	0.0234	0.0215	0.0382	0.0443	0.0681	0.0438	0.1166	0.0886
Ireland	0.0253	0.0278	0.0385	0.0502	0.1077	0.0419	0.1428	0.0763
England	0.0139	0.0244	0.0447	0.0406	0.0465	0.0358	0.1066	0.0609
Norway	0.0151	0.0935	0.1585	0.0966	0.0338	0.1851	0.2582	0.1886
Sweden.L	0.0078	0.0165	0.0149	0.0274	0.0215	0.0351	0.0533	0.0594
L. muta	0.04	0.0354	0.0629	0.0632	0.1042	0.0678	0.181	0.1203
Greenland.West	0.0341	0.06	0.0301	0.0867	0.0915	0.1193	0.1063	0.1722
Greenland.East	0.0357	0.0749	0.0422	0.147	0.1062	0.1488	0.1712	0.2908
Iceland	0.015	0.0138	0.0177	0.0272	0.0308	0.0267	0.0684	0.053
Pyrenees	0.0397	0.0231	0.0625	0.0419	0.0881	0.0375	0.1558	0.0673
Alps	0.067	0.0303	0.1755	0.0593	0.219	0.0457	0.4627	0.0867
Sweden.M	0.0082	0.0165	0.0179	0.0275	0.0215	0.0341	0.0576	0.0579
Svalbard	0.0803	0.0291	0.0943	0.053	0.1726	0.0627	0.2454	0.114

Table 1. Consolidated chart of offset values showing the average genomic offset scores for the various populations as calculated by both BayPass and LEA with all individuals included at K=10 using the full SNP dataset. Scores are broken up by climate dataset (CHELSA [CH] and WorldClim [WC]) and the concentration pathways (RCP 4.5 [45] and RCP 8.5 [85]).

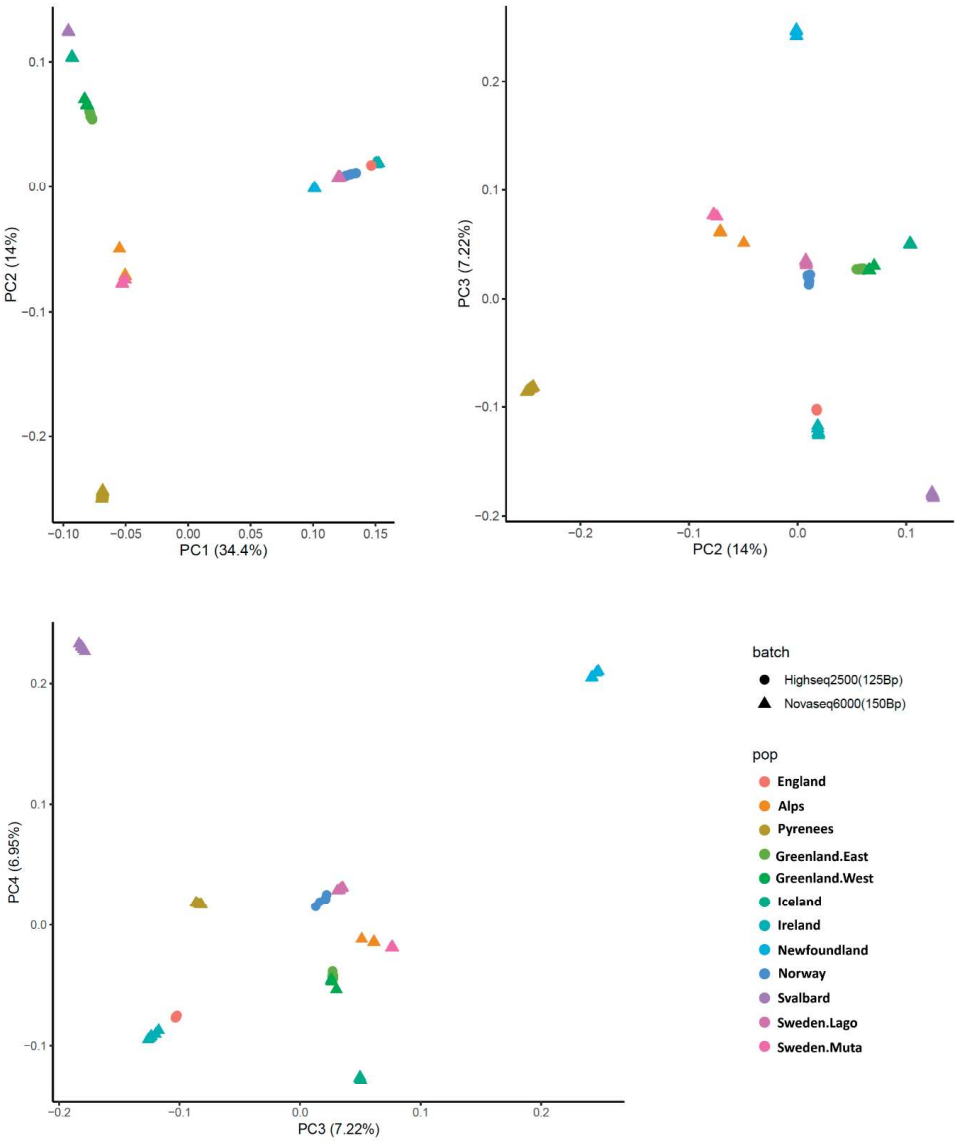

Figure S1. Three PCA plots where the shape of the population corresponds to which sequencing platform was used. This is to show that we have no obvious signs of batch effects in our data in spite of having two different read lengths and sequencing on two different machines with different chemistries. These plots were created using PLINK v1.90b7 and ggplot on the LD pruned VCF files. Importantly we see that groups parse along lines based on demography and geography as expected and don't appear to be separated based on sequencing batches.

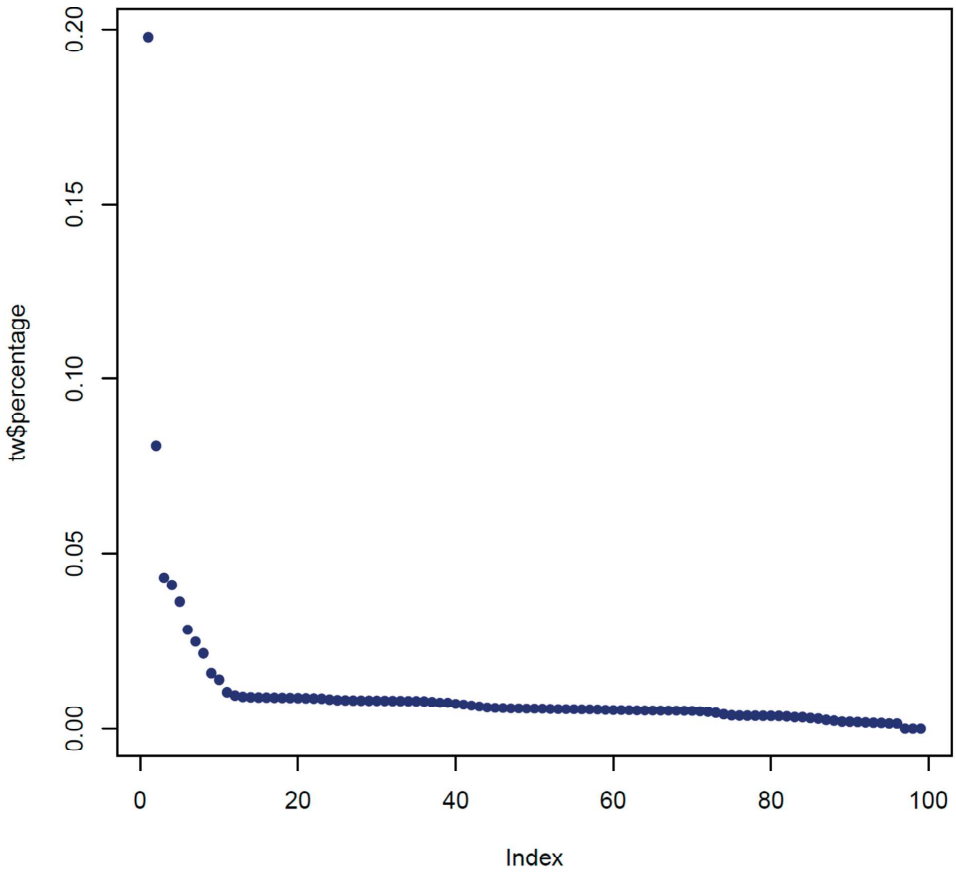

Figure S2. Tracy-Widom screeplot from 'LEA' validating a breakdown of K=10 populations for optimization of subsequent tests as suggested by Cattell's rule.

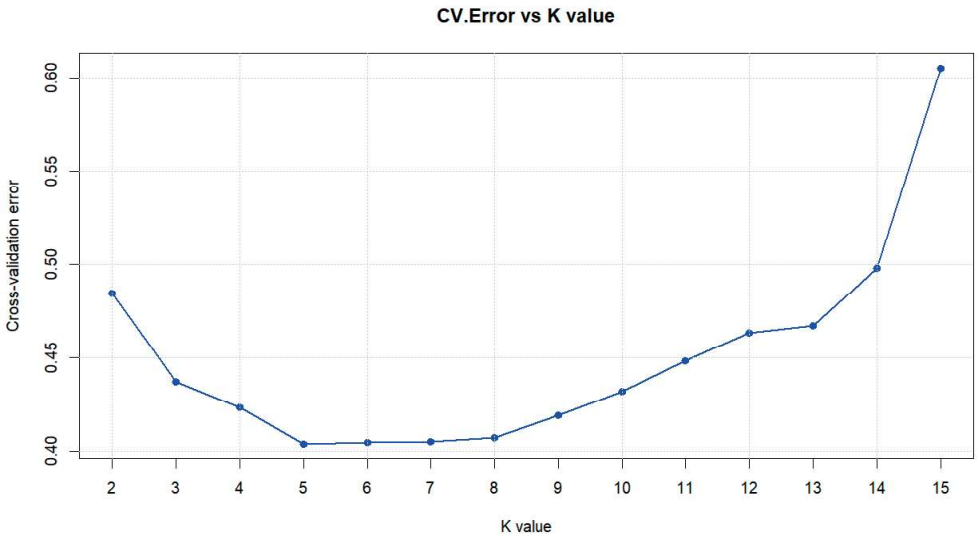

Figure S3. The cv.error from ADMIXTURE shows an optimal K value at 5 (0.40377) though the trend indication isn't very strong. K=10 still falls below the mean and median of discovered values.

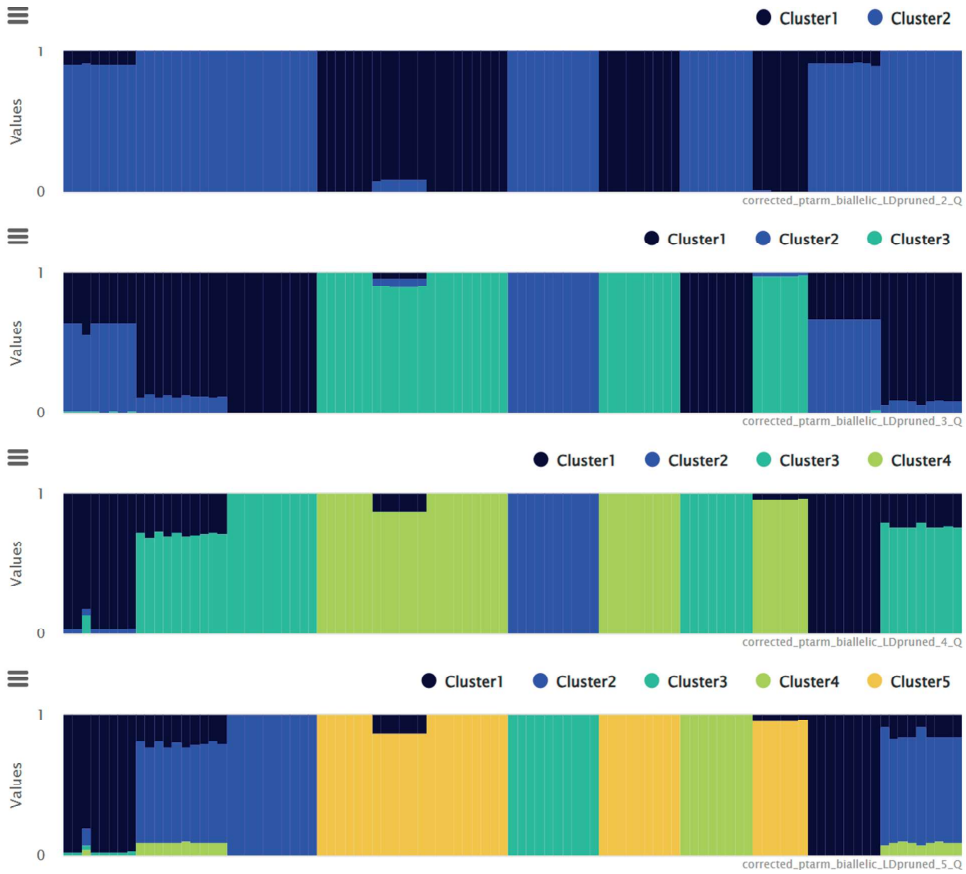

Figure S4. Raw plots for K = 2 through 5 as built from the PophelperShiny app using the output .Q files from ADMIXTURE. These indicate the genetic relatedness of populations with the order of 99 individuals (left to right) matching the order shown in table S1. At K=2 both species groups (*L. lagopus/scotica* and *L. muta*) break relatively cleanly.

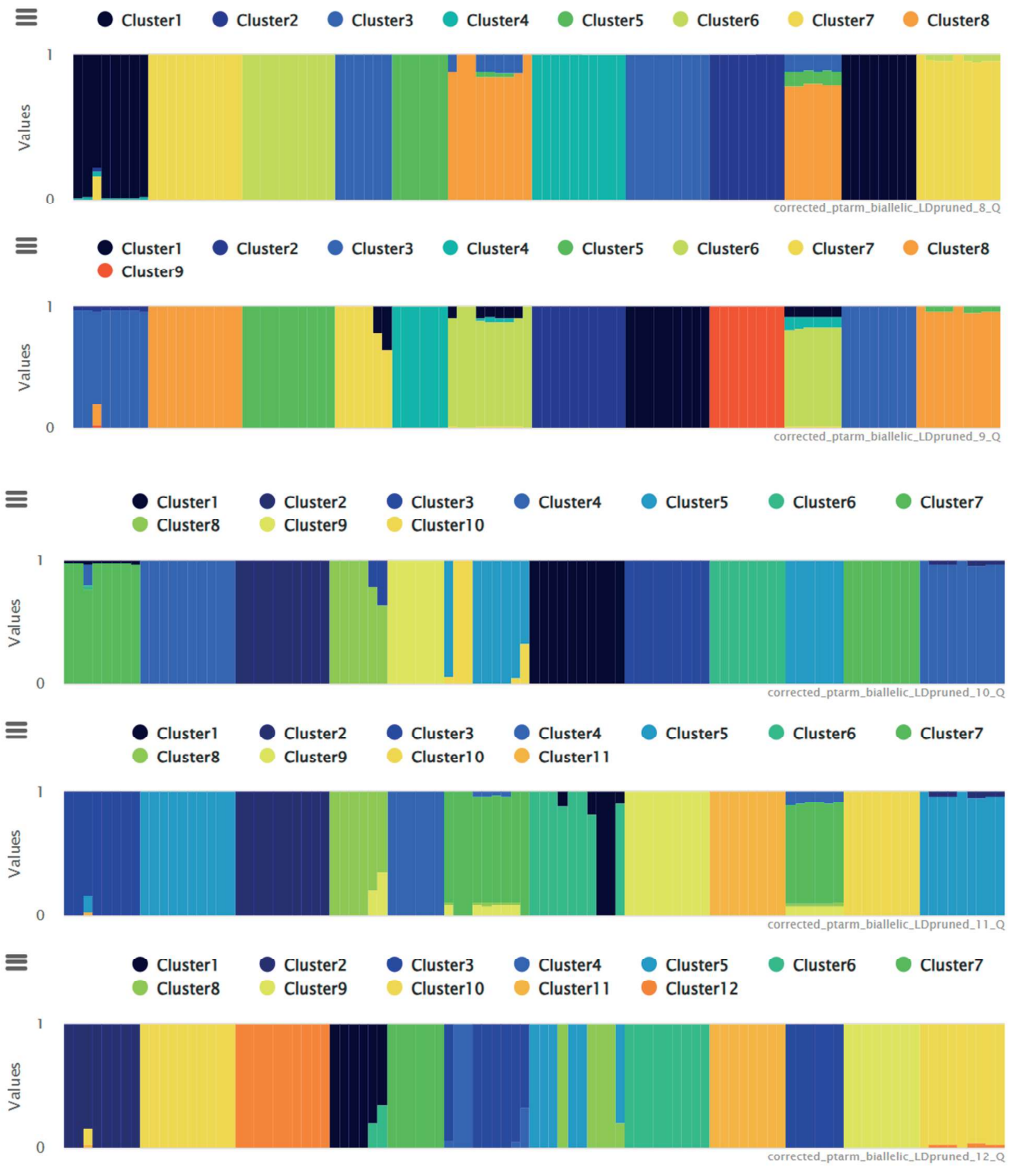

Figure S5. Raw plots for K = 8 through 12 as built from the PophelperShiny app using the output .Q files from ADMIXTURE. These indicate the genetic relatedness of populations with the order of 99 individuals (left to right) matching the order shown in table S1.

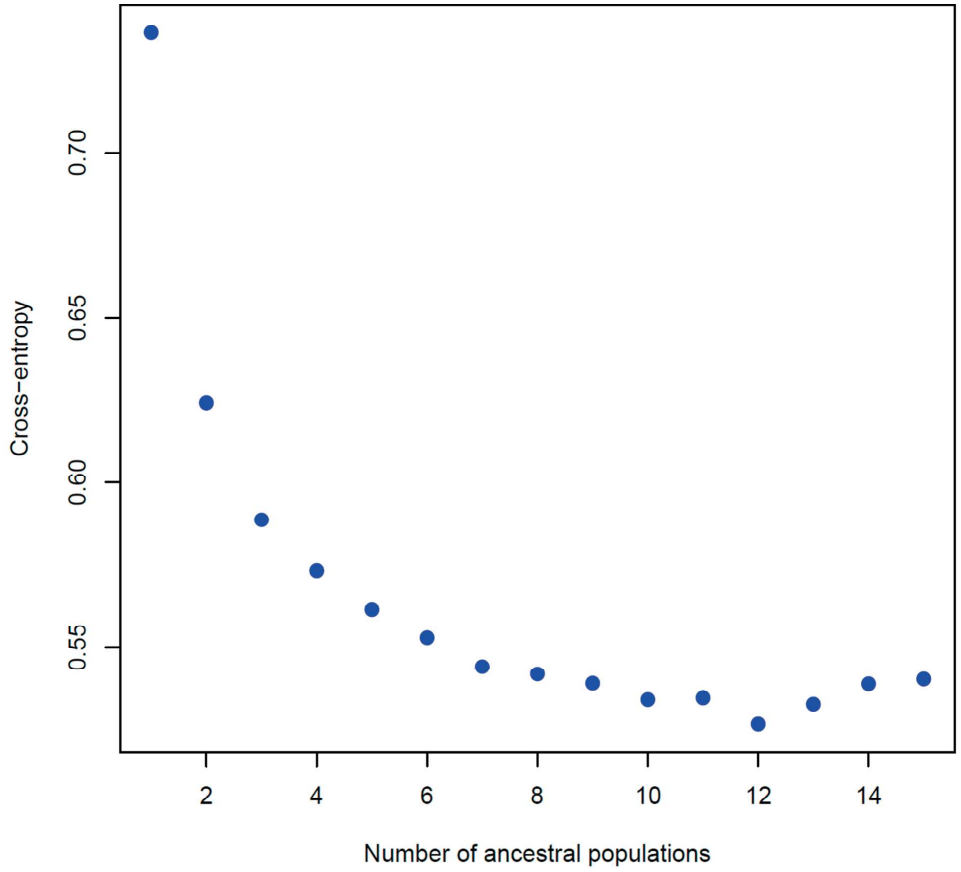

Figure S6. Least-squares Entropy output from LEA to estimate population structure by minimizing the discrepancy between observed and expected allele frequencies. Given a set of choices between K=2 and K=15 the lowest cross-entropy value appears to be at K=12.

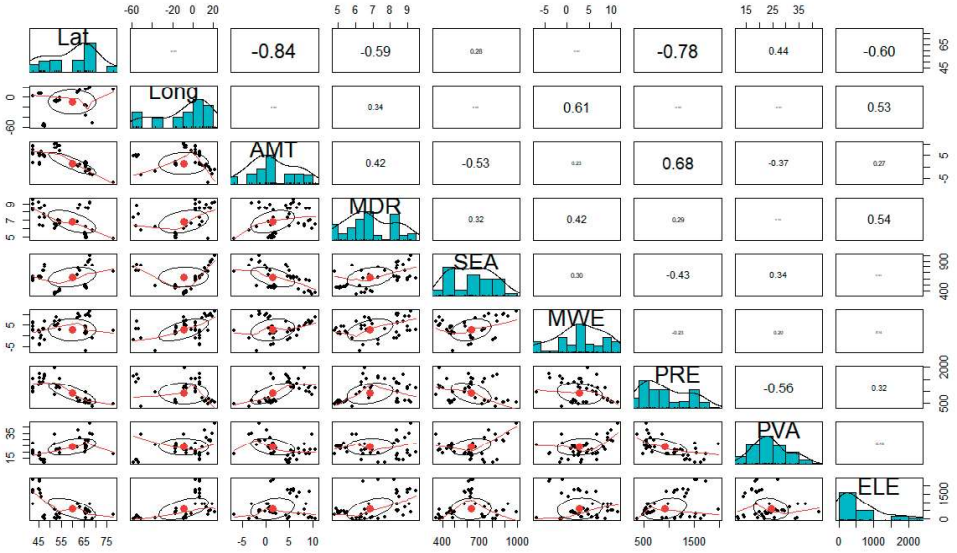

Figure S7. Pairs.panel assessment output from the 'psych' package in R showing collinearity scores between the final environmental terms with bioclimatic variables calculated from WorldClim along with latitude and longitude which were excluded from downstream analysis. Terms (left to right) are as follows: Latitude (Lat), Longitude (Long), Annual Mean Temperature (AMT), Mean Diurnal Temperature Range (MDR), Seasonality (SEA), Mean Temperature of the Wettest Quarter (MWE), Precipitation (PRE), Variability of Precipitation (PVA), and Elevation (ELE). Terms three through eight are derived from the 19 standard bioclimatic variables outlined in Fick and Hijmans (2017).

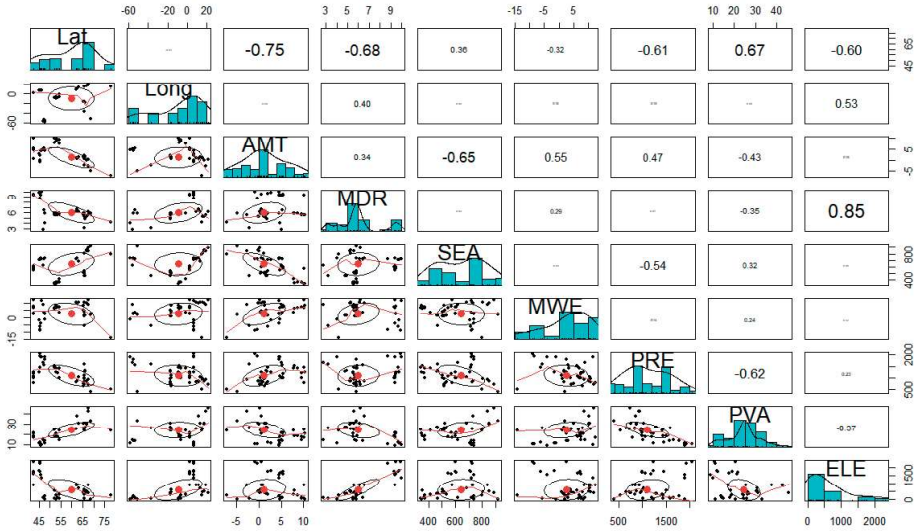

Figure S8. Pairs.panel assessment output from the 'psych' package in R showing collinearity scores between the final environmental terms with bioclimatic variables calculated from CHELSA along with latitude and longitude which were excluded from downstream analysis. Terms (left to right) are as follows: Latitude (Lat), Longitude (Long), Annual Mean Temperature (AMT), Mean Diurnal Temperature Range (MDR), Seasonality (SEA), Mean Temperature of the Wettest Quarter (MWE), Precipitation (PRE), Variability of Precipitation (PVA), and Elevation (ELE). Terms three through eight are derived from the 19 standard bioclimatic variables outlined in Fick and Hijmans (2017).

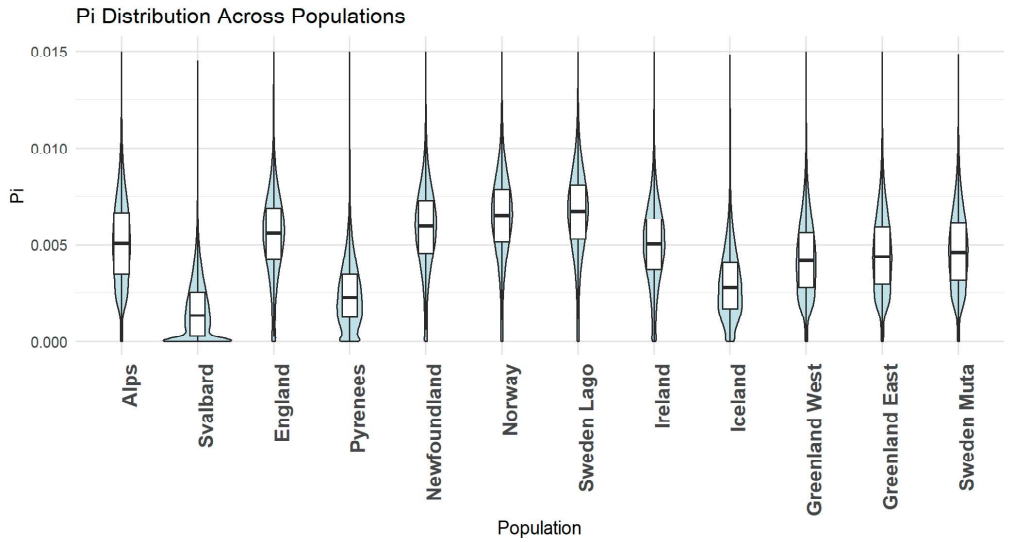

Figure S9. Violin plot developed using the 'ggplot' package showing comparative Pi (π) scores of *L. lagopus/scotica* and *L. muta* populations from the R package 'pixy'. Larger numbers indicate higher within population variation between individuals.

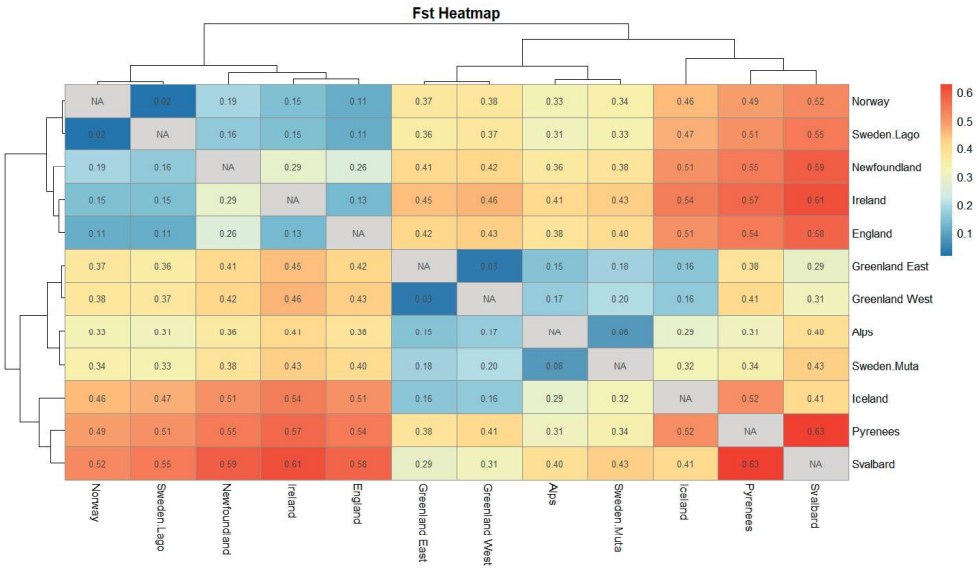

Figure S10. Heatmaps developed using the 'pheatmap' package (Kolde 2018) showing comparative Weir-Cockerham F_{ST} scores of *L. lagopus/scotica* and *L. muta* from the R package 'pixy'. Larger numbers indicate higher genetic differentiation between populations.

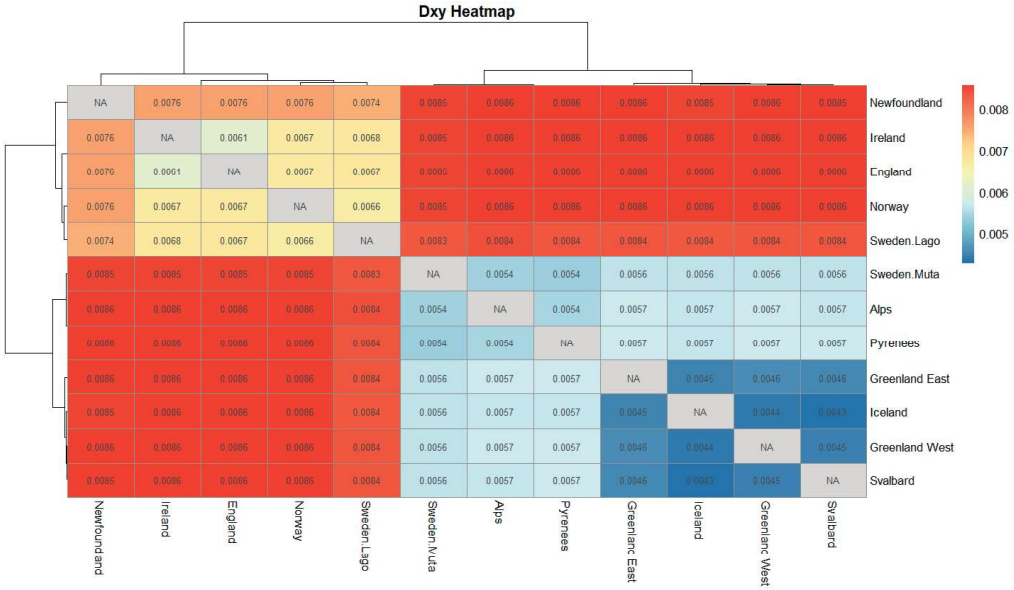

Figure S11. Heatmaps developed using the 'pheatmap' package (Kolde 2018) showing comparative Dxy scores of *L. lagopus/scotica* and *L. muta* populations from the R package 'pixy'. Larger numbers indicate higher absolute genetic divergence between populations.

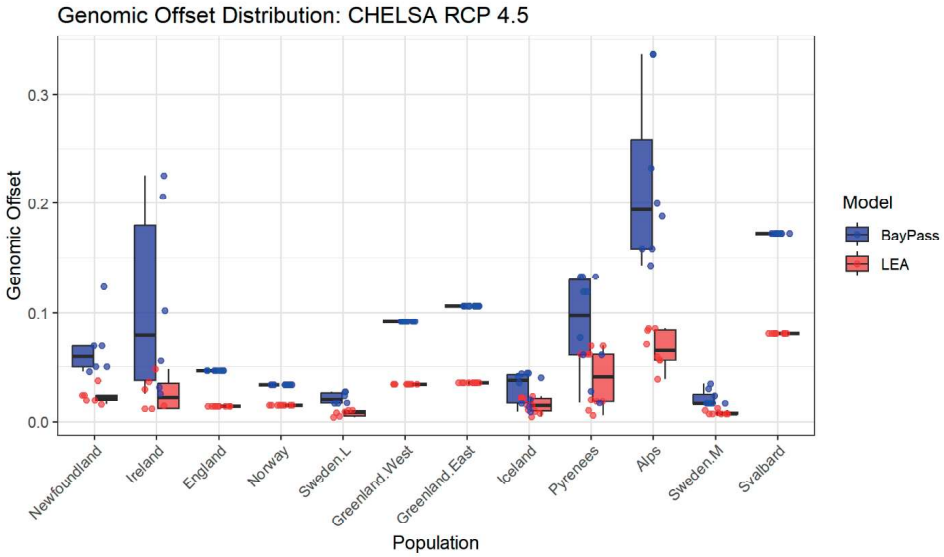

Figure S12. A boxplot showing the distribution of genetic offset among all populations using both BayPass and LEA under CHELSA's RCP 4.5 test.

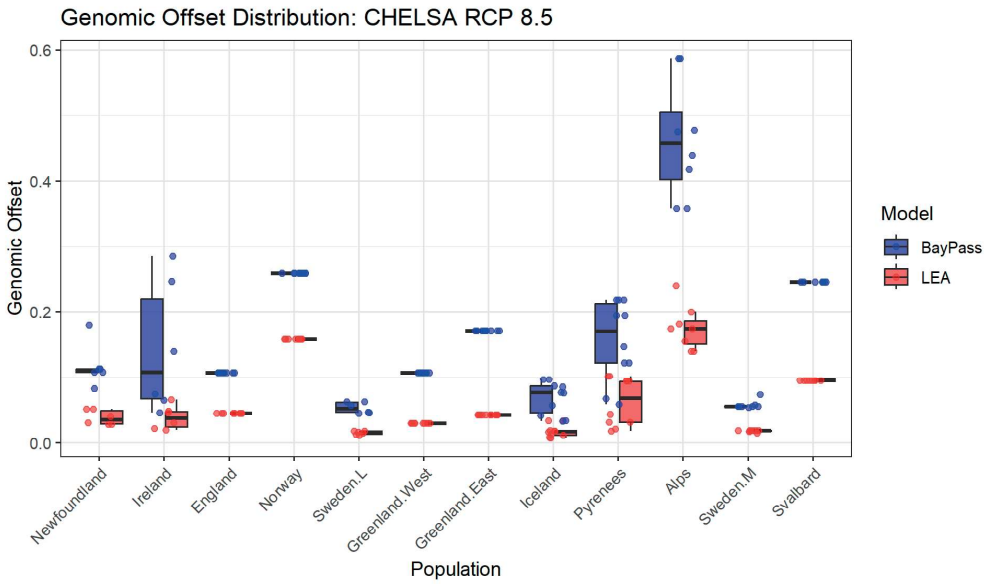

Figure S13. A boxplot showing the distribution of genetic offset among all populations using both BayPass and LEA under CHELSA's RCP 8.5 test.

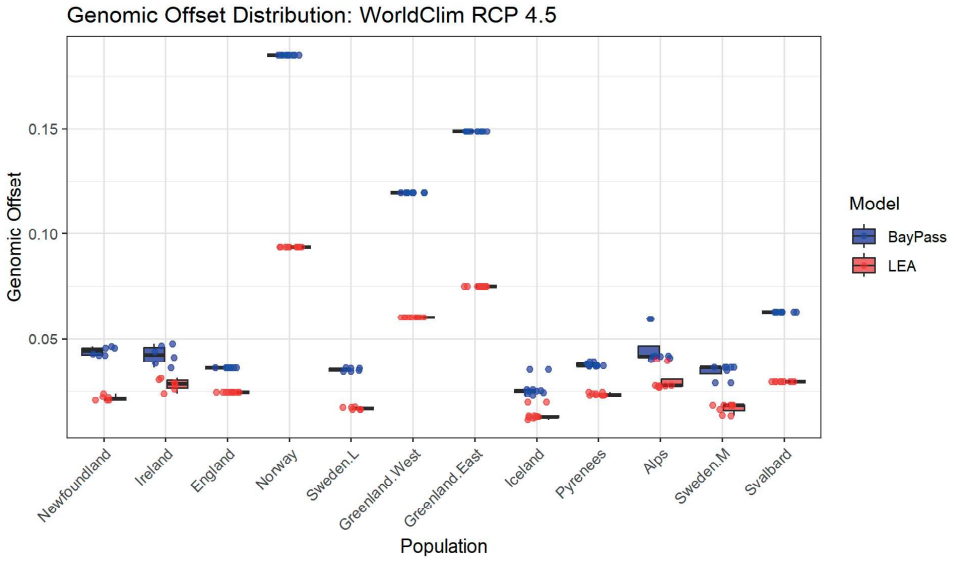

Figure S14. A boxplot showing the distribution of genetic offset among all populations using both BayPass and LEA under WorldClim's RCP 4.5 test.

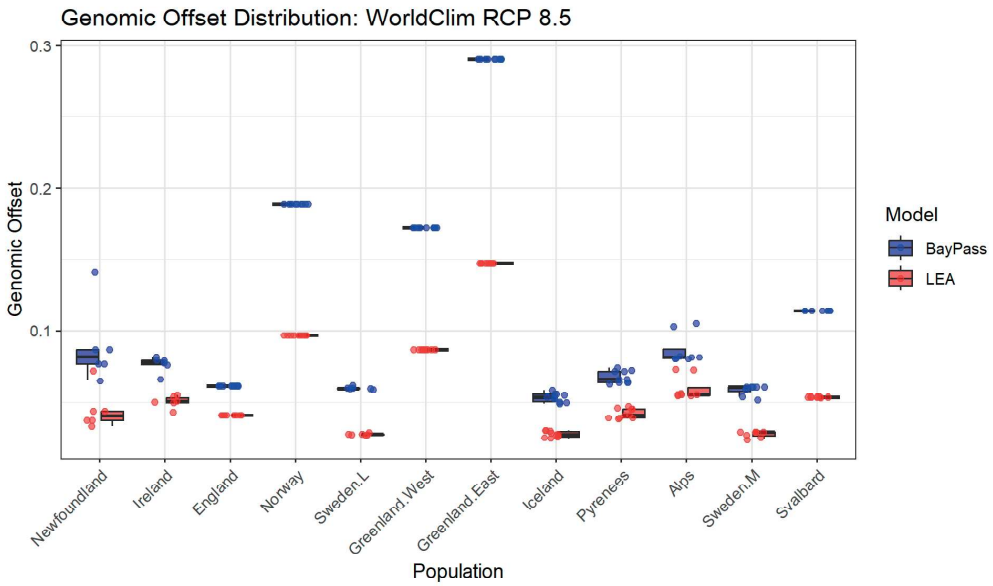

Figure S15. A boxplot showing the distribution of genetic offset among all populations using both BayPass and LEA under WorldClim's RCP 8.5 test.

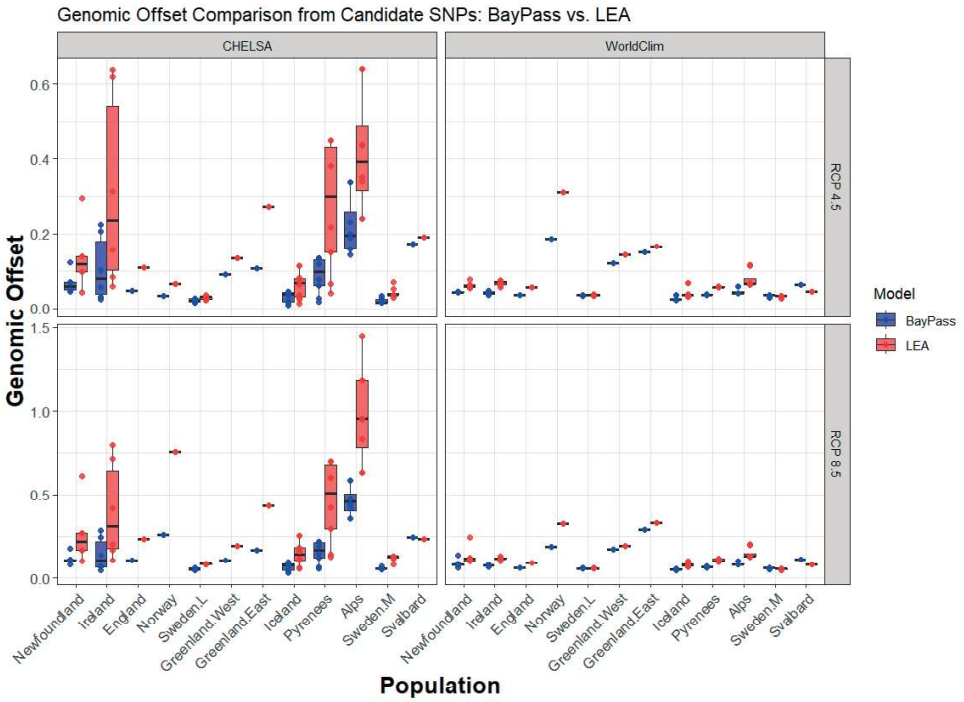

Figure S16. Figure showing four grouped box plots indicating the genomic offset scores for each of the study populations under various scenarios as calculated by BayPass (Blue) and LEA (Red) using the candidate SNP approach. For BayPass the calculations are based on 115,958 unique significantly associated SNPS for CHELSA and 104,706 SNPs for WorldClim. For LEA we used 37,781 unique significantly associated SNPS for CHELSA and 54,438 SNPs for WorldClim. The plots include the different climate datasets from CHELSA and WorldClim, along with the different relative concentration pathways tested at RCP 4.5 and RCP 8.5. Each plot is split with a line separating the *L. lagopus/scotica* group on the left, and the *L. muta* group on the right. Note that the Y axis for offset is scaled differently under the different concentration pathways. Candidate SNP approach offset scores for individuals under LEA and BayPass can be found in Table S9. Compare with Figure 4 in the main text.

PopID	PopName	Sample.no.	Population	Origin.Region	Spp	Lat	Long
Al1	F.alps	Al1	France.Alps	Alps	Lagopus muta	45.56163	6.821217
Al2	F.alps	Al2	France.Alps	Alps	Lagopus muta	46.04346	6.652426
Al3	F.alps	Al3	France.Alps	Alps	Lagopus muta	45.93038	6.899521
Al4	F.alps	Al4	France.Alps	Alps	Lagopus muta	45.33142	6.485849
Al5	F.alps	Al5	France.Alps	Alps	Lagopus muta	45.33142	6.485849
Al6	F.alps	Al6	France.Alps	Alps	Lagopus muta	45.3974	6.54638
Al7	F.alps	Al7	France.Alps	Alps	Lagopus muta	45.56163	6.821217
Al8	F.alps	Al8	France.Alps	Alps	Lagopus muta	45.20899	6.360879
Gr1	GrE	LMG-13-250	Greenland.East	Greenland	Lagopus muta	65.5677	-37.1816
Gr10	GrE	LMG-13-Unknown	Greenland.East	Greenland	Lagopus muta	65.5677	-37.1816
Gr2	GrE	LMG-13-251	Greenland.East	Greenland	Lagopus muta	65.5677	-37.1816
Gr3	GrE	LMG-13-252	Greenland.East	Greenland	Lagopus muta	65.5677	-37.1816
Gr4	GrE	LMG-13-253	Greenland.East	Greenland	Lagopus muta	65.5677	-37.1816
Gr5	GrE	LMG-13-254	Greenland.East	Greenland	Lagopus muta	65.5677	-37.1816
Gr6	GrE	LMG-13-255	Greenland.East	Greenland	Lagopus muta	65.5677	-37.1816
Gr7	GrE	LMG-13-256	Greenland.East	Greenland	Lagopus muta	65.5677	-37.1816
Gr8	GrE	LMG-13-257	Greenland.East	Greenland	Lagopus muta	65.5677	-37.1816
Gr9	GrE	LMG-13-260	Greenland.East	Greenland	Lagopus muta	65.5677	-37.1816
Ice1	Iceland	LM-17-034	Iceland	Iceland	Lagopus muta	65.75795	-16.727
Ice10	Iceland	LM-17-340	Iceland	Iceland	Lagopus muta	66.00331	-17.2572
Ice2	Iceland	LM-17-058	Iceland	Iceland	Lagopus muta	65.75345	-16.7298
Ice3	Iceland	LM-17-088	Iceland	Iceland	Lagopus muta	65.85958	-16.7828
Ice4	Iceland	LM-17-090	Iceland	Iceland	Lagopus muta	65.62938	-17.1781
Ice5	Iceland	LM-17-198	Iceland	Iceland	Lagopus muta	65.37151	-17.1038
Ice6	Iceland	LM-17-210	Iceland	Iceland	Lagopus muta	65.74774	-16.9532
Ice7	Iceland	LM-17-228	Iceland	Iceland	Lagopus muta	66.00882	-17.2483
Ice8	Iceland	LM-17-241	Iceland	Iceland	Lagopus muta	65.51888	-16.4735
Ice9	Iceland	LM-17-242	Iceland	Iceland	Lagopus muta	65.5589	-16.5868
Ire_Scot1	Ire_lago		Ireland	Clare	Lagopus lagopus/scotica	52.8487	-8.96077
Ire_Scot2	Ire_lago		Ireland	Monaghan	Lagopus lagopus/scotica	54.24568	-6.9686
Ire_Scot3	Ire_lago		Ireland	Cork	Lagopus lagopus/scotica	51.91316	-8.81721
Ire_Scot5	Ire_lago		Ireland	Roscommon	Lagopus lagopus/scotica	53.71771	-8.21519
Ire_Scot6	Ire_lago		Ireland	Knockmealdowns, Tipperary	Lagopus lagopus/scotica	52.22342	-7.92015
Ire_Scot9	Ire_lago		Ireland	Wicklow	Lagopus lagopus/scotica	53.0027	-6.34056
NL1001	Newfoundland	WIPT-NL-1001	Canada	Insular Newfoundland; Big Hill	Lagopus lagopus	47.81767	-55.2107
NL1003	Newfoundland	WIPT-NL-1003	Canada	Insular Newfoundland; Lebeach, Burin Peninsula	Lagopus lagopus	46.9892	-55.9412
NL1007	Newfoundland	WIPT-NL-1007	Canada	Insular Newfoundland; Lebeach, Burin Peninsula	Lagopus lagopus	46.9892	-55.9412
NL1010	Newfoundland	WIPT-NL-1010	Canada	Insular Newfoundland; Drift Inn, Lapoile	Lagopus lagopus	47.68105	-58.4052
NL1011	Newfoundland	WIPT-NL-1011	Canada	Insular Newfoundland	Lagopus lagopus	47.68105	-58.4052
NL1013	Newfoundland	WIPT-NL-1013	Canada	Insular Newfoundland; Hawke Hills	Lagopus lagopus	47.29027	-53.2265
Lago10	Norw_lago	Lago10	Norway	Dovre, Norway	Lagopus lagopus	62.22499	8.818492
Lago11	Norw_lago	Lago11	Norway	Dovre, Norway	Lagopus lagopus	62.22499	8.818492
Lago12	Norw_lago	Lago12	Norway	Dovre, Norway	Lagopus lagopus	62.22499	8.818492
Lago13	Norw_lago	Lago13	Norway	Dovre, Norway	Lagopus lagopus	62.22499	8.818492
Lago14	Norw_lago	Lago14	Norway	Dovre, Norway	Lagopus lagopus	62.22499	8.818492
Lago15	Norw_lago	Lago15	Norway	Dovre, Norway	Lagopus lagopus	62.22499	8.818492
Lago7	Norw_lago	Lago7	Norway	Dovre, Norway	Lagopus lagopus	62.22499	8.818492
Lago8	Norw_lago	Lago8	Norway	Dovre, Norway	Lagopus lagopus	62.22499	8.818492
Lago9	Norw_lago	Lago9	Norway	Dovre, Norway	Lagopus lagopus	62.22499	8.818492
Py1	F.pyrenees	Py1	France.Pyrenees	Pyrenees	Lagopus muta	42.54862	2.453245
Py10	F.pyrenees	Py10	France.Pyrenees	Pyrenees	Lagopus muta	42.37469	2.321872
Py2	F.pyrenees	Py2	France.Pyrenees	Pyrenees	Lagopus muta	42.53933	2.380971
Py3	F.pyrenees	Py3	France.Pyrenees	Pyrenees	Lagopus muta	42.48026	2.108093
Py4	F.pyrenees	Py4	France.Pyrenees	Pyrenees	Lagopus muta	42.54862	2.453245
Py5	F.pyrenees	Py5	France.Pyrenees	Pyrenees	Lagopus muta	42.54862	2.453245
Py6	F.pyrenees	Py6	France.Pyrenees	Pyrenees	Lagopus muta	42.53933	2.380971
Py7	F.pyrenees	Py7	France.Pyrenees	Pyrenees	Lagopus muta	42.4365	2.156962
Py8	F.pyrenees	Py8	France.Pyrenees	Pyrenees	Lagopus muta	42.4365	2.156962
Py9	F.pyrenees	Py9	France.Pyrenees	Pyrenees	Lagopus muta	42.42575	2.062733
Scot1	Eng_lago	Scot1	England	Feetham Yorkshire Dales, UK	Lagopus lagopus/scotica	54.37888	-2.06404
Scot2	Eng_lago	Scot2	England	Feetham Yorkshire Dales, UK	Lagopus lagopus/scotica	54.37888	-2.06404
Scot3	Eng_lago	Scot3	England	Feetham Yorkshire Dales, UK	Lagopus lagopus/scotica	54.37888	-2.06404
Scot4	Eng_lago	Scot4	England	Feetham Yorkshire Dales, UK	Lagopus lagopus/scotica	54.37888	-2.06404
Scot5	Eng_lago	Scot5	England	Gunnerside Yorkshire Dales, UK	Lagopus lagopus/scotica	54.37888	-2.06404
Scot6	Eng_lago	Scot6	England	Gunnerside Yorkshire Dales, UK	Lagopus lagopus/scotica	54.37888	-2.06404
Scot7	Eng_lago	Scot7	England	Gunnerside Yorkshire Dales, UK	Lagopus lagopus/scotica	54.37888	-2.06404
Scot8	Eng_lago	Scot8	England	Gunnerside Yorkshire Dales, UK	Lagopus lagopus/scotica	54.37888	-2.06404
Scot9	Eng_lago	Scot9	England	Gunnerside Yorkshire Dales, UK	Lagopus lagopus/scotica	54.37888	-2.06404
Sva115	Svalbard	Sva115	Svalbard	Svalbard	Lagopus muta	78.01667	15.11667
Sva126	Svalbard	Sva126	Svalbard	Svalbard	Lagopus muta	78.01667	15.11667
Sva129	Svalbard	Sva129	Svalbard	Svalbard	Lagopus muta	78.01667	15.11667
Sva188	Svalbard	Sva188	Svalbard	Svalbard	Lagopus muta	78.01667	15.11667
Sva294	Svalbard	Sva299	Svalbard	Svalbard	Lagopus muta	78.01667	15.11667
Sva296	Svalbard	SvaG194	Svalbard	Svalbard	Lagopus muta	78.01667	15.11667
Sva299	Svalbard	SvaG294	Svalbard	Svalbard	Lagopus muta	78.01667	15.11667
SvaG194	Svalbard	SvaG296	Svalbard	Svalbard	Lagopus muta	78.01667	15.11667
P25065_10	Swe_lago	JHGO089	Sweden.L	Sarta	Lagopus lagopus	66.86	17.0282
P25065_10	Swe_lago	JHGO090	Sweden.L	Miekak	Lagopus lagopus	66.8534	16.9395
P25065_10	Swe_lago	JHGO091	Sweden.L	Miekak	Lagopus lagopus	66.8534	16.9395
P25065_10	Swe_lago	JHGO092	Sweden.L	Liukajanratj	Lagopus lagopus	67.5848	20.9619
P25065_10	Swe_lago	JHGO093	Sweden.L	Kaisejåkkå	Lagopus lagopus	67.6983	18.9633
P25065_10	Swe_lago	JHGO094	Sweden.L	Kaiseloukta	Lagopus lagopus	67.6904	18.9675
JHGO938	Swe_muta	JHGO938	Sweden.M	Jamtland	Lagopus muta	62.48101	11.08505
JHGO939	Swe_muta	JHGO939	Sweden.M	Jamtland	Lagopus muta	62.48101	11.08505
JHGO940	Swe_muta	JHGO940	Sweden.M	Jamtland	Lagopus muta	62.48101	11.08505
JHGO941	Swe_muta	JHGO941	Sweden.M	Jamtland	Lagopus muta	62.48101	11.08505
JHGO942	Swe_muta	JHGO942	Sweden.M	Jamtland	Lagopus muta	62.48101	11.08505
Jam35	Swe_muta	Jam35	Sweden.M	Jamtland	Lagopus muta	63.15925	12.98671
P25065_11	Swe_muta	JHGO095	Sweden.M	Sarta	Lagopus muta	66.86	17.0282
P25065_11	Swe_muta	JHGO150	Sweden.M	Tärnaby	Lagopus muta	65.7407	15.3654
JHGO281	GrW	JHGO281	Greenland.West	Qasigiannguit Greenland, DK	Lagopus muta	68.82308	-51.3156
JHGO283	GrW	JHGO283	Greenland.West	Qasigiannguit Greenland, DK	Lagopus muta	68.82308	-51.3156
JHGO293	GrW	JHGO293	Greenland.West	Qasigiannguit Greenland, DK	Lagopus muta	68.82308	-51.3156
Muta1	GrW	Muta_1	Greenland.West	Qasigiannguit Greenland, DK	Lagopus muta	68.82308	-51.3156
Muta2	GrW	Muta_2	Greenland.West	Qasigiannguit Greenland, DK	Lagopus muta	68.82308	-51.3156
Muta3	GrW	Muta_3	Greenland.West	Qasigiannguit Greenland, DK	Lagopus muta	68.82308	-51.3156
Muta4	GrW	Muta_4	Greenland.West	Qasigiannguit Greenland, DK	Lagopus muta	68.82308	-51.3156
Muta5	GrW	Muta_5	Greenland.West	Qasigiannguit Greenland, DK	Lagopus muta	68.82308	-51.3156
Muta6	GrW	Muta_6	Greenland.West	Qasigiannguit Greenland, DK	Lagopus muta	68.82308	-51.3156

Table S1. Sampling coordinates and ID details for sequenced samples used in the study. Sequences from Ireland (Ire_Scot 1,2,3,5,6 and 9) were obtained from Walsh et al. (in review), those from Norway (Lago7-15) and from England (Scot1-9) were obtained from Kozma et al. (2019).

Variable Code	Variable Name	Source Dataset(s)	Justification for Retention
AMT	Annual Mean Temperature	CHELSA & WorldClim	Low collinearity (r<±0.7) with other variables.
MDR	Mean Diurnal Range	CHELSA & WorldClim	Low collinearity. Retained in CHELSA despite r=0.85 with ELE to test distinct thermal stress factors.
SEA	Temperature Seasonality	CHELSA & WorldClim	Low collinearity.
MWE	Mean Temp. of Wettest Quarter	CHELSA & WorldClim	Low collinearity.
PRE	Annual Precipitation	CHELSA & WorldClim	Low collinearity. Retained in WorldClim despite r=0.72 with AMT due to ecological importance.
PVA	Precipitation Seasonality	CHELSA & WorldClim	Low collinearity.
ELE	Elevation	GeoNames	Low collinearity. Included as a key non-climatic geographic variable.

Table S2. Summary table of the environmental variables used in the final analyses and reasons for their retention.

Population	Individual	Depth of coverage	% mapped reads
Alps (m)	Al1	37.21	98.8
Alps (m)	Al2	37.58	99.3
Alps (m)	Al3	35.06	99.1
Alps (m)	Al4	43.76	99.5
Alps (m)	Al5	42.61	98.3
Alps (m)	Al6	38.78	99.1
Alps (m)	Al7	44.81	98.3
Alps (m)	Al8	47.13	99.0
E.Greenland (m)	Gr10	35.00	99.3
E.Greenland (m)	Gr1	35.00	99.2
E.Greenland (m)	Gr2	40.77	98.9
E.Greenland (m)	Gr3	30.00	98.9
E.Greenland (m)	Gr4	34.54	98.9
E.Greenland (m)	Gr5	45.53	99.2
E.Greenland (m)	Gr6	31.64	98.4
E.Greenland (m)	Gr7	27.30	99.3
E.Greenland (m)	Gr8	35.00	98.4
E.Greenland (m)	Gr9	40.27	97.3
W.Greenland (m)	JHGO281	35.00	99.3
W.Greenland (m)	JHGO283	35.00	99.1
W.Greenland (m)	JHGO293	41.72	99.4
W.Greenland (m)	Muta1	28.78	99.0
W.Greenland (m)	Muta2	29.28	99.0
W.Greenland (m)	Muta3	29.46	98.7
W.Greenland (m)	Muta4	32.59	99.0
W.Greenland (m)	Muta5	30.75	99.2
W.Greenland (m)	Muta6	29.55	99.0
Iceland (m)	Ice10	43.16	97.5
Iceland (m)	Ice1	35.00	98.1
Iceland (m)	Ice2	35.00	97.5
Iceland (m)	Ice3	35.00	98.1
Iceland (m)	Ice4	34.53	98.3
Iceland (m)	Ice5	35.00	98.6
Iceland (m)	Ice6	35.00	98.1
Iceland (m)	Ice7	42.40	98.0
Iceland (m)	Ice8	35.00	97.7
Iceland (m)	Ice9	38.42	98.1
Ireland (l)	Ire_Scot1	26.21	98.6
Ireland (l)	Ire_Scot2	27.87	98.8
Ireland (l)	Ire_Scot3	27.28	98.9
Ireland (l)	Ire_Scot5	29.21	98.9
Ireland (l)	Ire_Scot6	26.13	99.1
Ireland (l)	Ire_Scot9	25.95	98.0
Sweden (m)	Jam35	39.42	98.5
Sweden (m)	JHGO938	35.00	98.7
Sweden (m)	JHGO939	36.90	98.9
Sweden (m)	JHGO940	34.44	98.5
Sweden (m)	JHGO941	43.11	98.6
Sweden (m)	JHGO942	34.92	98.3
Norway (l)	Lago10	28.15	99.0
Norway (l)	Lago11	33.67	98.9
Norway (l)	Lago12	30.40	98.9
Norway (l)	Lago13	26.72	98.9
Norway (l)	Lago14	31.85	98.9
Norway (l)	Lago15	31.24	98.5
Norway (l)	Lago7	33.24	99.0
Norway (l)	Lago8	31.90	98.8
Norway (l)	Lago9	29.70	99.0
Newfoundland (l)	NL1001	33.23	99.1
Newfoundland (l)	NL1003	43.18	99.2
Newfoundland (l)	NL1007	27.41	99.1
Newfoundland (l)	NL1010	49.09	99.1
Newfoundland (l)	NL1011	42.66	99.1
Newfoundland (l)	NL1013	33.48	99.2
Sweden (l)	P25065_104_S4	28.94	99.2
Sweden (l)	P25065_105_S5	20.05	99.0
Sweden (l)	P25065_106_S6	15.96	99.0
Sweden (l)	P25065_107_S7	18.72	99.4
Sweden (l)	P25065_108_S8	18.55	99.4
Sweden (l)	P25065_109_S9	22.58	99.1
Sweden (m)	P25065_110_S10	20.13	99.2
Sweden (m)	P25065_111_S11	20.00	98.9
Pyrenees (m)	Py10	42.91	98.3
Pyrenees (m)	Py1	45.36	99.0
Pyrenees (m)	Py2	48.55	98.8
Pyrenees (m)	Py3	42.07	99.3
Pyrenees (m)	Py4	48.85	98.7
Pyrenees (m)	Py5	30.80	99.4
Pyrenees (m)	Py6	35.00	99.3
Pyrenees (m)	Py7	43.76	99.1
Pyrenees (m)	Py8	35.00	99.5
Pyrenees (m)	Py9	35.00	99.1
England (l)	Scot1	28.74	98.6
England (l)	Scot2	29.00	98.6
England (l)	Scot3	33.29	99.0
England (l)	Scot4	28.53	98.4
England (l)	Scot5	32.03	98.6
England (l)	Scot6	28.83	98.9
England (l)	Scot7	29.45	98.6
England (l)	Scot8	29.70	98.9
England (l)	Scot9	31.09	98.6
Svalbard (m)	Sva115	42.77	99.2
Svalbard (m)	Sva126	43.92	99.2
Svalbard (m)	Sva129	32.13	99.2
Svalbard (m)	Sva188	50.13	99.2
Svalbard (m)	Sva294	35.00	99.2
Svalbard (m)	Sva296	25.64	99.2
Svalbard (m)	Sva299	40.17	99.2
Svalbard (m)	SvaG194	38.99	99.2

Table S3. Summary of generated sequence data showing depth of coverage for each individual sequences along with alignment to the reference genome.

Worldclim	AMT	MDR	SEA	MWE	PRE	PVA	ELE	Total
All_baypass_BF10	23366	6697	6430	16870	18140	8544	24659	104706
muta_baypass_BF10	4455	5	12538	20125	18401	20625	16399	92548
lagopus_baypass_BF10	17162	1874	9176	18968	21131	5575	21866	95752
Chelsa	**AMT**	**MDR**	**SEA**	**MWE**	**PRE**	**PVA**	**ELE**	**Total**
All_baypass_BF10	23274	17071	8540	23954	10377	8015	24727	115958
muta_baypass_BF10	10899	17399	10572	19066	12223	21293	16258	107710
lagopus_baypass_BF10	18790	8893	16194	26769	21169	5725	21881	119421

Table S4. Chart detailing the number of SNPs very significantly ($p < 0.001$) associated with environmental terms under BayPass for each climate model and various population breakdowns (All individuals; *L. muta* only, and *L. lagopus/scotica* only). Environmental terms (left to right) are as follows: Annual Mean Temperature (AMT), Mean Diurnal Temperature Range (MDR), Seasonality (SEA), Mean Temperature of the Wettest Quarter (MWE), Precipitation (PRE), Variability of Precipitation (PVA), and Elevation (ELE).

	WorldClim			CHELSA		
	WC_All	**WC_Lago**	**WC_Muta**	**CH_All**	**CH_Lago**	**CH_Muta**
AMT	4609	11827	26299	5684	29096	32133
MDR	2427	19739	32863	18188	63801	64248
SEA	12130	15574	13984	7605	29373	43974
MWE	5606	26065	17389	12564	72921	37935
PRE	9943	14289	31791	8787	51105	39256
PVA	4429	10280	23287	7913	29608	43069
ELE	5871	10866	22926	9936	30529	36272
TOTAL:	45015	108640	168539	70677	306433	296887
UNIQUE:	37781	78799	96460	54438	135486	163411

Table S5. Chart detailing the number of SNPs very significantly ($q < 0.05$) associated with environmental terms under LEA for each climate model and various population breakdowns (All individuals; *L. lagopus/scotica* only, and *L. muta* only). Environmental terms (left to right) are as follows: Annual Mean Temperature (AMT), Mean Diurnal Temperature Range (MDR), Seasonality (SEA), Mean Temperature of the Wettest Quarter (MWE), Precipitation (PRE), Variability of Precipitation (PVA), and Elevation (ELE). Note that the totals aren't reflective of unique SNPs and instead show the sum of significant associations for all sites, as some sites may have been identified as significantly correlated for multiple terms as described in the results section. Instead, the total number of unique correlated sites are shown on the bottom row.

Molecular Function	term_id	adjusted_p_value
protein binding	GO:0005515	5.36225E-38
binding	GO:0005488	1.90759E-25
catalytic activity, acting on a protein	GO:0140096	3.81629E-23
ion binding	GO:0043167	4.85222E-23
anion binding	GO:0043168	5.93313E-23
ATP binding	GO:0005524	1.99408E-21
adenyl ribonucleotide binding	GO:0032559	5.20989E-21
small molecule binding	GO:0036094	1.4633E-20
adenyl nucleotide binding	GO:0030554	8.42454E-20
carbohydrate derivative binding	GO:0097367	1.53816E-19
ribonucleotide binding	GO:0032553	3.41205E-19
purine ribonucleotide binding	GO:0032555	4.05782E-19
purine ribonucleoside triphosphate binding	GO:0035639	5.40313E-19
heterocyclic compound binding	GO:1901363	1.07811E-18
purine nucleotide binding	GO:0017076	2.45206E-18
enzyme binding	GO:0019899	3.89107E-18
nucleoside phosphate binding	GO:1901265	5.5756E-18
nucleotide binding	GO:0000166	6.79462E-18
catalytic activity	GO:0003824	1.42511E-17
protein-macromolecule adaptor activity	GO:0030674	1.65918E-15
transferase activity	GO:0016740	1.78647E-15
protein kinase activity	GO:0004672	4.15879E-14
molecular adaptor activity	GO:0060090	1.32524E-13
phosphotransferase activity, alcohol group as acceptor	GO:0016773	2.92095E-13
kinase activity	GO:0016301	6.41122E-13
Biological Processes		
developmental process	GO:0032502	4.31445E-40
positive regulation of cellular process	GO:0048522	5.59011E-37
anatomical structure development	GO:0048856	7.85189E-36
positive regulation of biological process	GO:0048518	1.44482E-35
localization	GO:0051179	4.2052E-34
cellular localization	GO:0051641	8.98201E-32
regulation of cell communication	GO:0010646	4.84533E-28
regulation of signaling	GO:0023051	6.60025E-28
intracellular signal transduction	GO:0035556	1.69275E-26
multicellular organism development	GO:0007275	9.13355E-26
cellular component organization	GO:0016043	3.25575E-25
cellular component organization or biogenesis	GO:0071840	2.18601E-24
regulation of response to stimulus	GO:0048583	7.93665E-24
macromolecule localization	GO:0033036	3.07554E-23
biological regulation	GO:0065007	8.97443E-23
system development	GO:0048731	1.14977E-22
establishment of localization	GO:0051234	1.44264E-22
cellular macromolecule localization	GO:0070727	1.47504E-22
regulation of signal transduction	GO:0009966	2.03555E-22
protein localization	GO:0008104	2.34813E-22
cellular developmental process	GO:0048869	1.51627E-21
cell differentiation	GO:0030154	1.51627E-21
transport	GO:0006810	1.78967E-21
regulation of cellular component organization	GO:0051128	2.88289E-21
regulation of biological process	GO:0050789	6.95843E-20
Cellular Component		
cytoplasm	GO:0005737	5.10098E-81
intracellular anatomical structure	GO:0005622	3.68132E-42
membrane-bounded organelle	GO:0043227	9.41674E-37
cytosol	GO:0005829	3.66376E-36
cell junction	GO:0030054	5.77295E-36
organelle	GO:0043226	4.20559E-32
intracellular membrane-bounded organelle	GO:0043231	5.14143E-31
nucleoplasm	GO:0005654	4.10991E-30
cellular anatomical structure	GO:0110165	2.3797E-29
endomembrane system	GO:0012505	2.39991E-29
synapse	GO:0045202	1.02588E-26
cell projection	GO:0042995	3.32121E-26
intracellular organelle	GO:0043229	4.46265E-25
plasma membrane bounded cell projection	GO:0120025	1.96779E-24
cell periphery	GO:0071944	1.81946E-22
glutamatergic synapse	GO:0098978	1.71171E-18
plasma membrane region	GO:0098590	7.36143E-18
plasma membrane	GO:0005886	1.12432E-17
nuclear lumen	GO:0031981	2.33125E-14
microtubule cytoskeleton	GO:0015630	9.62615E-14
Golgi apparatus	GO:0005794	1.25086E-13
intracellular organelle lumen	GO:0070013	9.14492E-13
membrane-enclosed lumen	GO:0031974	9.14492E-13
organelle lumen	GO:0043233	9.14492E-13
postsynapse	GO:0098794	1.36846E-12

Table S6a. List of the 25 most significantly associated gene ontology terms for three categories as determined from running all individuals in LEA against the CHELSA environmental dataset. Full tables available for download.

Molecular Function	term_id	adjusted_p_value
protein binding	GO:0005515	1.41331E-34
anion binding	GO:0043168	8.54654E-28
carbohydrate derivative binding	GO:0097367	1.05193E-25
ribonucleotide binding	GO:0032553	5.50102E-25
purine ribonucleotide binding	GO:0032555	5.50698E-25
purine ribonucleoside triphosphate binding	GO:0035639	6.12466E-25
purine nucleotide binding	GO:0017076	1.17589E-24
heterocyclic compound binding	GO:1901363	1.90885E-23
ion binding	GO:0043167	2.90643E-23
nucleoside phosphate binding	GO:1901265	3.0698E-23
nucleotide binding	GO:0000166	3.22713E-23
ATP binding	GO:0005524	4.7718E-23
adenyl ribonucleotide binding	GO:0032559	6.45101E-23
adenyl nucleotide binding	GO:0030554	2.44918E-22
catalytic activity, acting on a protein	GO:0140096	9.13094E-22
binding	GO:0005488	2.50418E-21
small molecule binding	GO:0036094	1.08759E-20
catalytic activity	GO:0003824	2.05836E-19
ribonucleoside triphosphate phosphatase activity	GO:0017111	5.88634E-17
pyrophosphatase activity	GO:0016462	2.83745E-16
activity, acting on acid anhydrides, in phosphorus-containing a	GO:0016818	3.32751E-16
hydrolase activity, acting on acid anhydrides	GO:0016817	4.33885E-16
nucleoside-triphosphatase regulator activity	GO:0060589	5.50076E-15
GTPase regulator activity	GO:0030695	5.50076E-15
enzyme binding	GO:0019899	3.6743E-13
Biological Processes		
developmental process	GO:0032502	2.7938E-39
anatomical structure development	GO:0048856	1.57591E-37
positive regulation of biological process	GO:0048518	7.33872E-35
positive regulation of cellular process	GO:0048522	3.49947E-32
multicellular organism development	GO:0007275	4.8862E-29
biological regulation	GO:0065007	4.18871E-27
localization	GO:0051179	2.51759E-26
system development	GO:0048731	4.99911E-26
regulation of cell communication	GO:0010646	2.71363E-24
regulation of biological process	GO:0050789	4.41845E-24
cellular developmental process	GO:0048869	4.5162E-24
cell differentiation	GO:0030154	4.5162E-24
multicellular organismal process	GO:0032501	5.28858E-24
regulation of signaling	GO:0023051	6.35774E-24
intracellular signal transduction	GO:0035556	1.10027E-23
regulation of response to stimulus	GO:0048583	4.10539E-23
cellular component organization	GO:0016043	3.01987E-22
cellular component organization or biogenesis	GO:0071840	1.59539E-20
nervous system development	GO:0007399	2.75138E-20
regulation of cellular component organization	GO:0051128	1.68193E-19
intracellular signaling cassette	GO:0141124	2.81825E-19
regulation of cellular process	GO:0050794	3.3521E-19
cellular localization	GO:0051641	3.94917E-19
regulation of signal transduction	GO:0009966	5.51451E-19
protein modification process	GO:0036211	1.94296E-16
Cellular Component		
cytoplasm	GO:0005737	2.01157E-62
cytosol	GO:0005829	1.09751E-35
cell junction	GO:0030054	4.9339E-33
intracellular anatomical structure	GO:0005622	9.31638E-33
nucleoplasm	GO:0005654	6.09598E-29
synapse	GO:0045202	4.35383E-25
membrane-bounded organelle	GO:0043227	1.27353E-24
cellular anatomical structure	GO:0110165	1.99607E-24
cell projection	GO:0042995	7.89346E-23
plasma membrane bounded cell projection	GO:0120025	6.39749E-22
organelle	GO:0043226	1.61323E-21
endomembrane system	GO:0012505	2.28285E-21
intracellular membrane-bounded organelle	GO:0043231	4.07327E-19
glutamatergic synapse	GO:0098978	6.84656E-18
cell periphery	GO:0071944	1.09387E-16
nuclear lumen	GO:0031981	2.63497E-15
intracellular organelle	GO:0043229	2.6423E-15
plasma membrane	GO:0005886	4.25236E-14
organelle lumen	GO:0043233	2.80688E-13
membrane-enclosed lumen	GO:0031974	2.80688E-13
intracellular organelle lumen	GO:0070013	2.80688E-13
neuron projection	GO:0043005	1.09178E-12
postsynapse	GO:0098794	4.27926E-12
plasma membrane region	GO:0098590	6.81503E-12
dendrite	GO:0030425	1.46344E-11

Table S6b. List of the 25 most significantly associated gene ontology terms for three categories as determined from running all individuals in LEA against the WorldClim environmental dataset. Full tables available for download.

Molecular Function	term_id	adjusted_p_value
protein binding	GO:0005515	3.42574E-51
binding	GO:0005488	1.92669E-30
catalytic activity, acting on a protein	GO:0140096	7.11422E-18
ATP binding	GO:0005524	4.27117E-17
adenyl ribonucleotide binding	GO:0032559	1.51101E-16
adenyl nucleotide binding	GO:0030554	1.03429E-15
ion binding	GO:0043167	2.37978E-15
protein-macromolecule adaptor activity	GO:0030674	5.29280E-15
anion binding	GO:0043168	1.06158E-14
molecular adaptor activity	GO:0060090	2.35363E-14
small molecule binding	GO:0036094	8.75639E-14
enzyme binding	GO:0019899	1.73978E-13
ATP-dependent activity	GO:0140657	6.54455E-13
purine ribonucleoside triphosphate binding	GO:0035639	1.93476E-12
nucleotide binding	GO:0000166	1.94702E-12
nucleoside phosphate binding	GO:1901265	1.94702E-12
purine ribonucleotide binding	GO:0032555	2.15254E-12
heterocyclic compound binding	GO:1901363	3.72217E-12
carbohydrate derivative binding	GO:0097367	4.36569E-12
purine nucleotide binding	GO:0017076	6.05051E-12
ribonucleotide binding	GO:0032553	6.65328E-12
nucleoside-triphosphatase regulator activity	GO:0060589	1.70173E-11
GTPase regulator activity	GO:0030695	1.70173E-11
transferase activity	GO:0016740	3.02652E-09
cytoskeletal protein binding	GO:0008092	6.70977E-09
Biological processes		
localization	GO:0051179	1.27556E-35
developmental process	GO:0032502	1.58577E-34
multicellular organismal process	GO:0032501	2.82041E-33
cellular localization	GO:0051641	1.06785E-30
positive regulation of biological process	GO:0048518	1.39649E-30
anatomical structure development	GO:0048856	8.91227E-30
regulation of cell communication	GO:0010646	5.20676E-29
cellular component organization	GO:0016043	1.99811E-28
regulation of signaling	GO:0023051	2.16120E-28
cellular component organization or biogenesis	GO:0071840	7.56556E-28
positive regulation of cellular process	GO:0048522	4.28370E-27
establishment of localization	GO:0051234	1.51944E-25
regulation of response to stimulus	GO:0048583	2.84393E-23
transport	GO:0006810	1.74983E-22
intracellular signal transduction	GO:0035556	5.09552E-22
macromolecule modification	GO:0043412	1.40675E-21
regulation of signal transduction	GO:0009966	1.48370E-21
protein modification process	GO:0036211	5.99203E-21
macromolecule localization	GO:0033036	6.79073E-21
multicellular organism development	GO:0007275	1.25412E-20
protein localization	GO:0008104	7.87352E-20
cellular macromolecule localization	GO:0070727	8.55591E-20
biological regulation	GO:0065007	2.71855E-19
cellular developmental process	GO:0048869	4.42549E-19
cell differentiation	GO:0030154	4.42549E-19
Cellular component		
cytoplasm	GO:0005737	2.50043E-63
cell periphery	GO:0071944	4.88139E-34
intracellular anatomical structure	GO:0005622	2.11969E-33
plasma membrane	GO:0005886	1.25967E-31
endomembrane system	GO:0012505	2.95304E-29
cytosol	GO:0005829	1.21888E-28
membrane-bounded organelle	GO:0043227	7.17354E-28
intracellular membrane-bounded organelle	GO:0043231	1.35435E-24
cellular anatomical entity	GO:0110165	5.63538E-23
nucleoplasm	GO:0005654	3.66384E-22
organelle	GO:0043226	5.95716E-22
cell junction	GO:0030054	1.23930E-21
synapse	GO:0045202	1.01613E-18
intracellular organelle	GO:0043229	1.20007E-18
cell projection	GO:0042995	1.75460E-15
plasma membrane bounded cell projection	GO:0120025	1.06901E-14
glutamatergic synapse	GO:0098978	3.64349E-14
nuclear lumen	GO:0031981	1.07553E-11
plasma membrane region	GO:0098590	2.27763E-11
organelle lumen	GO:0043233	5.41826E-11
intracellular organelle lumen	GO:0070013	5.41826E-11
membrane-enclosed lumen	GO:0031974	5.41826E-11
Golgi apparatus	GO:0005794	3.72255E-10
intracellular vesicle	GO:0097708	2.95009E-09
microtubule cytoskeleton	GO:0015630	4.23504E-09

Table S6c. List of the 25 most significantly associated gene ontology terms for three categories as determined from running all individuals in BayPass against the CHELSA environmental dataset. Full tables available for download.

Molecular Function	term_id	adjusted_p_value
protein binding	GO:0005515	9.79821E-54
binding	GO:0005488	3.98402E-30
ATP binding	GO:0005524	2.78347E-19
adenyl ribonucleotide binding	GO:0032559	9.04901E-19
catalytic activity, acting on a protein	GO:0140096	3.94982E-18
adenyl nucleotide binding	GO:0030554	1.47999E-17
enzyme binding	GO:0019899	8.11416E-16
ion binding	GO:0043167	3.96034E-15
anion binding	GO:0043168	1.52711E-14
small molecule binding	GO:0036094	2.10889E-13
protein kinase activity	GO:0004672	4.14269E-13
protein-macromolecule adaptor activity	GO:0030674	2.74291E-12
purine ribonucleotide binding	GO:0032555	7.48351E-12
nucleotide binding	GO:0000166	8.25641E-12
nucleoside phosphate binding	GO:1901265	8.25641E-12
purine ribonucleoside triphosphate binding	GO:0035639	8.30841E-12
phosphotransferase activity, alcohol group as acceptor	GO:0016773	1.14914E-11
ribonucleotide binding	GO:0032553	1.36469E-11
heterocyclic compound binding	GO:1901363	2.13929E-11
molecular adaptor activity	GO:0060090	2.54484E-11
purine nucleotide binding	GO:0017076	3.05755E-11
transferase activity	GO:0016740	4.65230E-11
carbohydrate derivative binding	GO:0097367	7.21694E-11
kinase activity	GO:0016301	7.66120E-11
protein serine/threonine kinase activity	GO:0004674	1.46365E-10
Biological processes		
developmental process	GO:0032502	6.80486E-35
localization	GO:0051179	6.19656E-33
anatomical structure development	GO:0048856	1.30918E-32
multicellular organismal process	GO:0032501	2.55691E-30
positive regulation of biological process	GO:0048518	2.53275E-29
cellular localization	GO:0051641	6.68141E-28
positive regulation of cellular process	GO:0048522	3.96863E-27
macromolecule modification	GO:0043412	6.88978E-26
protein modification process	GO:0036211	2.13731E-24
cellular component organization	GO:0016043	9.66278E-24
intracellular signal transduction	GO:0035556	1.79451E-23
regulation of cell communication	GO:0010646	1.88143E-23
regulation of signaling	GO:0023051	4.08773E-23
cellular component organization or biogenesis	GO:0071840	5.26476E-23
multicellular organism development	GO:0007275	1.09521E-22
establishment of localization	GO:0051234	2.78052E-22
regulation of response to stimulus	GO:0048583	1.36123E-20
transport	GO:0006810	1.93508E-20
cell differentiation	GO:0030154	3.87756E-20
cellular developmental process	GO:0048869	3.87756E-20
system development	GO:0048731	1.05262E-19
regulation of cellular component organization	GO:0051128	8.87330E-19
macromolecule localization	GO:0033036	1.02927E-17
vesicle-mediated transport	GO:0016192	1.04903E-17
regulation of signal transduction	GO:0009966	2.72919E-17
Cellular component		
cytoplasm	GO:0005737	1.49728E-63
cell periphery	GO:0071944	1.80798E-33
intracellular anatomical structure	GO:0005622	1.33761E-31
plasma membrane	GO:0005886	1.61689E-30
cytosol	GO:0005829	1.68109E-27
membrane-bounded organelle	GO:0043227	1.83305E-26
endomembrane system	GO:0012505	2.04014E-24
nucleoplasm	GO:0005654	3.52402E-23
intracellular membrane-bounded organelle	GO:0043231	3.76173E-23
cell junction	GO:0030054	1.68421E-22
cellular anatomical entity	GO:0110165	2.59511E-19
organelle	GO:0043226	5.84418E-18
synapse	GO:0045202	8.53270E-18
intracellular organelle	GO:0043229	1.76777E-15
cell projection	GO:0042995	2.26772E-14
plasma membrane bounded cell projection	GO:0120025	1.53419E-13
glutamatergic synapse	GO:0098978	3.21196E-12
nuclear lumen	GO:0031981	8.43629E-12
Golgi apparatus	GO:0005794	1.12782E-11
membrane-enclosed lumen	GO:0031974	4.52417E-10
organelle lumen	GO:0043233	4.52417E-10
intracellular organelle lumen	GO:0070013	4.52417E-10
intracellular vesicle	GO:0097708	1.62949E-09
plasma membrane region	GO:0098590	2.44945E-09
cytoplasmic vesicle	GO:0031410	3.17711E-09

Table S6d. List of the most significantly associated gene ontology terms as determined from running BayPass against the WorldClim environmental dataset. Full tables available for download.

Table S7. Offset values as calculated via the standard run for LEA.

ID	Population	WorldClim45	WorldClim85	CHELSA45	CHELSA85
Al1	France.Alps	0.02741403	0.05442131	0.056354	0.139542
Al2	France.Alps	0.02653825	0.05510524	0.038926	0.240026
Al3	France.Alps	0.02710273	0.05592922	0.059434	0.154907
Al4	France.Alps	0.02739454	0.05491246	0.085111	0.173941
Al5	France.Alps	0.02739454	0.05491246	0.085111	0.173941
Al6	France.Alps	0.03991441	0.07217899	0.071034	0.181678
Al7	France.Alps	0.02741403	0.05442131	0.056354	0.139542
Al8	France.Alps	0.03931446	0.07279883	0.083351	0.200041
Gr1	Greenland.	0.07493673	0.14700769	0.035728	0.042199
Gr10	Greenland.	0.07493673	0.14700769	0.035728	0.042199
Gr2	Greenland.	0.07493673	0.14700769	0.035728	0.042199
Gr3	Greenland.	0.07493673	0.14700769	0.035728	0.042199
Gr4	Greenland.	0.07493673	0.14700769	0.035728	0.042199
Gr5	Greenland.	0.07493673	0.14700769	0.035728	0.042199
Gr6	Greenland.	0.07493673	0.14700769	0.035728	0.042199
Gr7	Greenland.	0.07493673	0.14700769	0.035728	0.042199
Gr8	Greenland.	0.07493673	0.14700769	0.035728	0.042199
Gr9	Greenland.	0.07493673	0.14700769	0.035728	0.042199
Ice1	Iceland	0.01926117	0.02985026	0.02124	0.033746
Ice10	Iceland	0.01283474	0.02804967	0.022962	0.01774
Ice2	Iceland	0.01926117	0.02985026	0.02124	0.033746
Ice3	Iceland	0.01236512	0.02531118	0.015376	0.018105
Ice4	Iceland	0.01194308	0.0269966	0.00925	0.0111
Ice5	Iceland	0.01261283	0.02466985	0.0109	0.007657
Ice6	Iceland	0.01314467	0.02742349	0.014807	0.012895
Ice7	Iceland	0.01323447	0.02973173	0.022119	0.018242
Ice8	Iceland	0.01133712	0.024556	0.004405	0.015876
Ice9	Iceland	0.01249362	0.02590361	0.007758	0.008047
Ire_Scot1	Ireland	0.02860794	0.05472803	0.014296	0.021774
Ire_Scot2	Ireland	0.02359372	0.04274342	0.011562	0.030175
Ire_Scot3	Ireland	0.02788191	0.04958554	0.036563	0.04573
Ire_Scot5	Ireland	0.02574141	0.0500212	0.011587	0.019515
Ire_Scot6	Ireland	0.0301978	0.05346599	0.029802	0.048051
Ire_Scot9	Ireland	0.03076966	0.05067408	0.048009	0.065583
NL1001	Canada	0.02039265	0.07144996	0.016014	0.030867
NL1003	Canada	0.02022856	0.03714308	0.024215	0.051174
NL1007	Canada	0.02022856	0.03714308	0.024215	0.051174
NL1010	Canada	0.0221291	0.04361995	0.019406	0.027734
NL1011	Canada	0.0221291	0.04361995	0.019406	0.027734
NL1013	Canada	0.02360406	0.03295119	0.037259	0.040339
Lago10	Norway	0.09354377	0.09658808	0.0151	0.158455
Lago11	Norway	0.09354377	0.09658808	0.0151	0.158455
Lago12	Norway	0.09354377	0.09658808	0.0151	0.158455
Lago13	Norway	0.09354377	0.09658808	0.0151	0.158455
Lago14	Norway	0.09354377	0.09658808	0.0151	0.158455
Lago15	Norway	0.09354377	0.09658808	0.0151	0.158455
Lago7	Norway	0.09354377	0.09658808	0.0151	0.158455
Lago8	Norway	0.09354377	0.09658808	0.0151	0.158455
Lago9	Norway	0.09354377	0.09658808	0.0151	0.158455
Py1	France.Pyre	0.02271141	0.03869781	0.061814	0.09318
Py10	France.Pyre	0.02240377	0.03820649	0.020091	0.043393
Py2	France.Pyre	0.02243427	0.04059714	0.069489	0.10063
Py3	France.Pyre	0.02433559	0.04703195	0.010102	0.02093
Py4	France.Pyre	0.02271141	0.03869781	0.061814	0.09318
Py5	France.Pyre	0.02271141	0.03869781	0.061814	0.09318
Py6	France.Pyre	0.02243427	0.04059714	0.069489	0.10063
Py7	France.Pyre	0.02369938	0.04518643	0.018561	0.031194
Py8	France.Pyre	0.02369938	0.04518643	0.018561	0.031194
Py9	France.Pyre	0.02429415	0.04571667	0.005708	0.017781
Scot1	England	0.02435286	0.0405584	0.013876	0.044682
Scot2	England	0.02435286	0.0405584	0.013876	0.044682
Scot3	England	0.02435286	0.0405584	0.013876	0.044682
Scot4	England	0.02435286	0.0405584	0.013876	0.044682
Scot5	England	0.02435286	0.0405584	0.013876	0.044682
Scot6	England	0.02435286	0.0405584	0.013876	0.044682
Scot7	England	0.02435286	0.0405584	0.013876	0.044682
Scot8	England	0.02435286	0.0405584	0.013876	0.044682
Scot9	England	0.02435286	0.0405584	0.013876	0.044682
Sva115	Svalbard	0.0291048	0.05302534	0.080267	0.094339
Sva126	Svalbard	0.0291048	0.05302534	0.080267	0.094339
Sva129	Svalbard	0.0291048	0.05302534	0.080267	0.094339
Sva188	Svalbard	0.0291048	0.05302534	0.080267	0.094339
Sva294	Svalbard	0.0291048	0.05302534	0.080267	0.094339
Sva296	Svalbard	0.0291048	0.05302534	0.080267	0.094339
Sva299	Svalbard	0.0291048	0.05302534	0.080267	0.094339
SvaG194	Svalbard	0.0291048	0.05302534	0.080267	0.094339
P25065_10	Sweden.L	0.01611752	0.02670525	0.008153	0.0148
P25065_10	Sweden.L	0.0159504	0.02712649	0.010431	0.018007
P25065_10	Sweden.L	0.0159504	0.02712649	0.010431	0.018007
P25065_10	Sweden.L	0.01715563	0.02850865	0.008973	0.015813
P25065_10	Sweden.L	0.01702214	0.02748333	0.004636	0.011177
P25065_10	Sweden.L	0.01702214	0.02748333	0.004104	0.011031
JHGO938	Sweden.M	0.01788932	0.028936	0.007096	0.018672
JHGO939	Sweden.M	0.01788932	0.028936	0.007096	0.018672
JHGO940	Sweden.M	0.01788932	0.028936	0.007096	0.018672
JHGO941	Sweden.M	0.01788932	0.028936	0.007096	0.018672
JHGO942	Sweden.M	0.01788932	0.028936	0.007096	0.018672
Jam35	Sweden.M	0.01345628	0.02491584	0.011976	0.016529
P25065_11	Sweden.M	0.01611752	0.02670525	0.008153	0.0148
P25065_11	Sweden.M	0.0131941	0.02353589	0.010169	0.018591
JHGO281	Greenland.I	0.06001624	0.08673705	0.034068	0.030072
JHGO283	Greenland.I	0.06001624	0.08673705	0.034068	0.030072
JHGO293	Greenland.I	0.06001624	0.08673705	0.034068	0.030072
Muta1	Greenland.I	0.06001624	0.08673705	0.034068	0.030072
Muta2	Greenland.I	0.06001624	0.08673705	0.034068	0.030072
Muta3	Greenland.I	0.06001624	0.08673705	0.034068	0.030072
Muta4	Greenland.I	0.06001624	0.08673705	0.034068	0.030072
Muta5	Greenland.I	0.06001624	0.08673705	0.034068	0.030072
Muta6	Greenland.I	0.06001624	0.08673705	0.034068	0.030072

All	CHELSA_45	CHELSA_85	Worldclim_45	Worldclim_85
France.Alps	0.1578022	0.3580908	0.04130191	0.0802551
France.Alps	0.1426628	0.4751807	0.03980129	0.0806046
France.Alps	0.1998586	0.4396516	0.04067282	0.0817743
France.Alps	0.3368945	0.5873362	0.0416541	0.0811651
France.Alps	0.3368945	0.5873362	0.0416541	0.0811651
France.Alps	0.2321592	0.4780732	0.05943327	0.1029792
France.Alps	0.1578022	0.3580908	0.04130191	0.0802551
France.Alps	0.1881708	0.4174614	0.05940442	0.1052215
Greenland.East	0.1061627	0.1711846	0.14876	0.2908113
Greenland.East	0.1061627	0.1711846	0.14876	0.2908113
Greenland.East	0.1061627	0.1711846	0.14876	0.2908113
Greenland.East	0.1061627	0.1711846	0.14876	0.29081132
Greenland.East	0.1061627	0.1711846	0.14876	0.29081132
Greenland.East	0.1061627	0.1711846	0.14876	0.29081132
Greenland.East	0.1061627	0.1711846	0.14876	0.29081132
Greenland.East	0.1061627	0.1711846	0.14876	0.29081132
Greenland.East	0.1061627	0.1711846	0.14876	0.29081132
Greenland.East	0.1061627	0.1711846	0.14876	0.29081132
Iceland	0.0443388	0.09636572	0.0350957	0.05549923
Iceland	0.04361735	0.07623829	0.02504391	0.05466116
Iceland	0.0443388	0.09636572	0.0350957	0.05549923
Iceland	0.0403264	0.08676732	0.02417353	0.04981208
Iceland	0.02000821	0.0572147	0.02351954	0.05311262
Iceland	0.01652213	0.03327616	0.02493265	0.04870002
Iceland	0.03564461	0.08498612	0.0255841	0.05321894
Iceland	0.04140997	0.07689249	0.02562285	0.05794741
Iceland	0.008763566	0.04174467	0.02260568	0.049389
Iceland	0.01333993	0.03395084	0.02486291	0.05221892
Ireland	0.05586866	0.07454031	0.04086915	0.07877337
Ireland	0.03155139	0.06489686	0.03580142	0.0656118
Ireland	0.2061771	0.24645	0.0435652	0.07728876
Ireland	0.02546517	0.04532697	0.03813494	0.07583643
Ireland	0.1019783	0.1401367	0.04609063	0.08162726
Ireland	0.2252888	0.2852009	0.04713512	0.07890133
Canada	0.04556233	0.08215264	0.04258226	0.1409815
Canada	0.05039416	0.1070804	0.04191621	0.07648525
Canada	0.05039416	0.1070804	0.04191621	0.07648525
Canada	0.06943841	0.1118337	0.04530899	0.08660987
Canada	0.06943841	0.1118337	0.04530899	0.08660987
Canada	0.1234531	0.1795769	0.04587354	0.06466999
Norway	0.03381944	0.258219	0.185148	0.18858739
Norway	0.03381944	0.258219	0.185148	0.18858739
Norway	0.03381944	0.258219	0.185148	0.18858739
Norway	0.03381944	0.258219	0.185148	0.18858739
Norway	0.03381944	0.258219	0.185148	0.18858739
Norway	0.03381944	0.258219	0.185148	0.18858739
Norway	0.03381944	0.258219	0.185148	0.18858739
Norway	0.03381944	0.258219	0.185148	0.18858739
Norway	0.03381944	0.258219	0.185148	0.18858739
France.Pyrenees	0.132844	0.2185082	0.03694046	0.06357312
France.Pyrenees	0.07702897	0.1468125	0.03674902	0.06237338
France.Pyrenees	0.1189478	0.1943384	0.03666891	0.06576901
France.Pyrenees	0.02761238	0.0670625	0.03910609	0.07398462
France.Pyrenees	0.132844	0.2185082	0.03694046	0.06357312
France.Pyrenees	0.132844	0.2185082	0.03694046	0.06357312
France.Pyrenees	0.1189478	0.1943384	0.03666891	0.06576901
France.Pyrenees	0.06113856	0.120769	0.03801489	0.07122017
France.Pyrenees	0.06113856	0.120769	0.03801489	0.07122017
France.Pyrenees	0.01742079	0.05843305	0.03908731	0.07206025
England	0.04653842	0.1066004	0.0358037	0.06094814
England	0.04653842	0.1066004	0.0358037	0.06094814
England	0.04653842	0.1066004	0.0358037	0.06094814
England	0.04653842	0.1066004	0.0358037	0.06094814
England	0.04653842	0.1066004	0.0358037	0.06094814
England	0.04653842	0.1066004	0.0358037	0.06094814
England	0.04653842	0.1066004	0.0358037	0.06094814
England	0.04653842	0.1066004	0.0358037	0.06094814
England	0.04653842	0.1066004	0.0358037	0.06094814
Svalbard	0.1726314	0.2453778	0.06265358	0.11402893
Svalbard	0.1726314	0.2453778	0.06265358	0.11402893
Svalbard	0.1726314	0.2453778	0.06265358	0.11402893
Svalbard	0.1726314	0.2453778	0.06265358	0.11402893
Svalbard	0.1726314	0.2453778	0.06265358	0.11402893
Svalbard	0.1726314	0.2453778	0.06265358	0.11402893
Svalbard	0.1726314	0.2453778	0.06265358	0.11402893
Svalbard	0.1726314	0.2453778	0.06265358	0.11402893
Sweden.L	0.02362336	0.05724266	0.03479757	0.05851758
Sweden.L	0.02719755	0.06273256	0.03428679	0.05939767
Sweden.L	0.02719755	0.06273256	0.03428679	0.05939767
Sweden.L	0.01734786	0.0448243	0.03595775	0.06144226
Sweden.L	0.01695604	0.04655991	0.03572325	0.05896607
Sweden.L	0.01666205	0.04591882	0.03572325	0.05896607
Sweden.M	0.01673638	0.0551895	0.03611316	0.06003043
Sweden.M	0.01673638	0.0551895	0.03611316	0.06003043
Sweden.M	0.01673638	0.0551895	0.03611316	0.06003043
Sweden.M	0.01673638	0.0551895	0.03611316	0.06003043
Sweden.M	0.01673638	0.0551895	0.03611316	0.06003043
Sweden.M	0.03000949	0.05340604	0.02863815	0.05339899
Sweden.M	0.02362336	0.05724266	0.03479757	0.05851758
Sweden.M	0.03440949	0.07385809	0.02874622	0.05129914
Greenland.West	0.09150675	0.1062597	0.1192945	0.17221485
Greenland.West	0.09150675	0.1062597	0.1192945	0.17221485
Greenland.West	0.09150675	0.1062597	0.1192945	0.17221485
Greenland.West	0.09150675	0.1062597	0.1192945	0.17221485
Greenland.West	0.09150675	0.1062597	0.1192945	0.17221485
Greenland.West	0.09150675	0.1062597	0.1192945	0.17221485
Greenland.West	0.09150675	0.1062597	0.1192945	0.17221485
Greenland.West	0.09150675	0.1062597	0.1192945	0.17221485
Greenland.West	0.09150675	0.1062597	0.1192945	0.17221485

Table S8. Offset values as calculated via the modified comparable run for BayPass.

Individual	Population	LEA_CH45	BayPass_CH45	LEA_WC45	BayPass_WC45	LEA_CH85	BayPass_CH85	LEA_WC85	BayPass_WC85
NL1001	Newfoundland	0.04338357	0.045562328	0.05443172	0.04258226	0.10346279	0.08215264	0.24551713	0.1409815
NL1003	Newfoundland	0.13722774	0.050394165	0.0557639	0.04191621	0.26932011	0.10708042	0.10338215	0.07648525
NL1007	Newfoundland	0.13722774	0.050394165	0.0557639	0.04191621	0.26932011	0.10708042	0.10338215	0.07648525
NL1010	Newfoundland	0.09675107	0.069438412	0.06375484	0.04530899	0.16966625	0.11183371	0.1251543	0.08960987
NL1011	Newfoundland	0.09675107	0.069438412	0.06375484	0.04530899	0.16966625	0.11183371	0.1251543	0.08960987
NL1013	Newfoundland	0.29467254	0.123453066	0.0775795	0.04587354	0.60852693	0.17957689	0.10627686	0.06466999
Ire_Scot1	Ireland	0.15690515	0.055868658	0.07407953	0.04086915	0.20512029	0.07454031	0.13442211	0.07877337
Ire_Scot2	Ireland	0.08298198	0.031551385	0.05658693	0.03580142	0.16954241	0.06489686	0.10086033	0.0656118
Ire_Scot3	Ireland	0.61861335	0.20617714	0.06748431	0.0435652	0.71388121	0.24644999	0.11626668	0.07728876
Ire_Scot5	Ireland	0.05992116	0.025465172	0.06085897	0.03813494	0.1079139	0.04532697	0.11942402	0.07583643
Ire_Scot6	Ireland	0.31303891	0.101978315	0.0728786	0.04609063	0.42027564	0.14013672	0.12522361	0.08162726
Ire_Scot9	Ireland	0.63677438	0.225288846	0.07313671	0.04713512	0.79250433	0.28520089	0.12018486	0.07890133
Scot 1	England	0.10939272	0.046538423	0.05574829	0.0358037	0.23304975	0.10660045	0.09283731	0.06094814
Scot 2	England	0.10939272	0.046538423	0.05574829	0.0358037	0.23304975	0.10660045	0.09283731	0.06094814
Scot 3	England	0.10939272	0.046538423	0.05574829	0.0358037	0.23304975	0.10660045	0.09283731	0.06094814
Scot 4	England	0.10939272	0.046538423	0.05574829	0.0358037	0.23304975	0.10660045	0.09283731	0.06094814
Scot 5	England	0.10939272	0.046538423	0.05574829	0.0358037	0.23304975	0.10660045	0.09283731	0.06094814
Scot 6	England	0.10939272	0.046538423	0.05574829	0.0358037	0.23304975	0.10660045	0.09283731	0.06094814
Scot 7	England	0.10939272	0.046538423	0.05574829	0.0358037	0.23304975	0.10660045	0.09283731	0.06094814
Scot 8	England	0.10939272	0.046538423	0.05574829	0.0358037	0.23304975	0.10660045	0.09283731	0.06094814
Scot 9	England	0.10939272	0.046538423	0.05574829	0.0358037	0.23304975	0.10660045	0.09283731	0.06094814
Lago10	Norway	0.06615602	0.033819439	0.31093278	0.18514799	0.75423183	0.25821897	0.3248472	0.18858739
Lago11	Norway	0.06615602	0.033819439	0.31093278	0.18514799	0.75423183	0.25821897	0.3248472	0.18858739
Lago12	Norway	0.06615602	0.033819439	0.31093278	0.18514799	0.75423183	0.25821897	0.3248472	0.18858739
Lago13	Norway	0.06615602	0.033819439	0.31093278	0.18514799	0.75423183	0.25821897	0.3248472	0.18858739
Lago14	Norway	0.06615602	0.033819439	0.31093278	0.18514799	0.75423183	0.25821897	0.3248472	0.18858739
Lago15	Norway	0.06615602	0.033819439	0.31093278	0.18514799	0.75423183	0.25821897	0.3248472	0.18858739
Lago7	Norway	0.06615602	0.033819439	0.31093278	0.18514799	0.75423183	0.25821897	0.3248472	0.18858739
Lago8	Norway	0.06615602	0.033819439	0.31093278	0.18514799	0.75423183	0.25821897	0.3248472	0.18858739
Lago9	Norway	0.06615602	0.033819439	0.31093278	0.18514799	0.75423183	0.25821897	0.3248472	0.18858739
P25065_10	Sweden.L	0.03034925	0.023623357	0.03484468	0.03479757	0.08076604	0.05724266	0.05612818	0.05851758
P25065_10	Sweden.L	0.03123087	0.027197552	0.03465234	0.03428679	0.0876571	0.06273256	0.05642507	0.05939767
P25065_10	Sweden.L	0.03123087	0.027197552	0.03465234	0.03428679	0.0876571	0.06273256	0.05642507	0.05939767
P25065_10	Sweden.L	0.03678641	0.017347858	0.0378891	0.03595775	0.08988613	0.0448243	0.05979359	0.06144226
P25065_10	Sweden.L	0.02340813	0.016956044	0.03828529	0.03572325	0.08406244	0.04655991	0.05936813	0.05896607
P25065_10	Sweden.L	0.0225806	0.016662051	0.03828529	0.03572325	0.08466198	0.04591882	0.05936813	0.05896607
JHGO281	Greenland.West	0.13388101	0.091506746	0.14273991	0.11929445	0.19556626	0.10625967	0.19383049	0.17221485
JHGO283	Greenland.West	0.13388101	0.091506746	0.14273991	0.11929445	0.19556626	0.10625967	0.19383049	0.17221485
JHGO293	Greenland.West	0.13388101	0.091506746	0.14273991	0.11929445	0.19556626	0.10625967	0.19383049	0.17221485
Muta1	Greenland.West	0.13388101	0.091506746	0.14273991	0.11929445	0.19556626	0.10625967	0.19383049	0.17221485
Muta2	Greenland.West	0.13388101	0.091506746	0.14273991	0.11929445	0.19556626	0.10625967	0.19383049	0.17221485
Muta3	Greenland.West	0.13388101	0.091506746	0.14273991	0.11929445	0.19556626	0.10625967	0.19383049	0.17221485
Muta4	Greenland.West	0.13388101	0.091506746	0.14273991	0.11929445	0.19556626	0.10625967	0.19383049	0.17221485
Muta5	Greenland.West	0.13388101	0.091506746	0.14273991	0.11929445	0.19556626	0.10625967	0.19383049	0.17221485
Muta6	Greenland.West	0.13388101	0.091506746	0.14273991	0.11929445	0.19556626	0.10625967	0.19383049	0.17221485
Gr1	Greenland.East	0.27142172	0.106162657	0.16561928	0.14875997	0.43525571	0.1711846	0.33121266	0.29081132
Gr10	Greenland.East	0.27142172	0.106162657	0.16561928	0.14875997	0.43525571	0.1711846	0.33121266	0.29081132
Gr2	Greenland.East	0.27142172	0.106162657	0.16561928	0.14875997	0.43525571	0.1711846	0.33121266	0.29081132
Gr3	Greenland.East	0.27142172	0.106162657	0.16561928	0.14875997	0.43525571	0.1711846	0.33121266	0.29081132
Gr4	Greenland.East	0.27142172	0.106162657	0.16561928	0.14875997	0.43525571	0.1711846	0.33121266	0.29081132
Gr5	Greenland.East	0.27142172	0.106162657	0.16561928	0.14875997	0.43525571	0.1711846	0.33121266	0.29081132
Gr6	Greenland.East	0.27142172	0.106162657	0.16561928	0.14875997	0.43525571	0.1711846	0.33121266	0.29081132
Gr7	Greenland.East	0.27142172	0.106162657	0.16561928	0.14875997	0.43525571	0.1711846	0.33121266	0.29081132
Gr8	Greenland.East	0.27142172	0.106162657	0.16561928	0.14875997	0.43525571	0.1711846	0.33121266	0.29081132
Gr9	Greenland.East	0.27142172	0.106162657	0.16561928	0.14875997	0.43525571	0.1711846	0.33121266	0.29081132
Ice1	Iceland	0.11271748	0.044338802	0.0673996	0.0350957	0.25595164	0.09636572	0.10210217	0.05549223
Ice10	Iceland	0.08050419	0.043617346	0.03733689	0.02504391	0.17046993	0.07623829	0.08279656	0.0546616
Ice2	Iceland	0.11271748	0.044338802	0.0673996	0.0350957	0.25595164	0.09636572	0.10210217	0.05549223
Ice3	Iceland	0.07206154	0.040326403	0.03686458	0.02417353	0.18572736	0.08676732	0.07388095	0.04981208
Ice4	Iceland	0.03085766	0.020008212	0.03337712	0.02351954	0.11149182	0.0572147	0.07535666	0.05311262
Ice5	Iceland	0.02529837	0.016522134	0.03438061	0.02493265	0.05792363	0.03327616	0.06668177	0.04870002
Ice6	Iceland	0.06248216	0.035644611	0.03815477	0.0255841	0.12008724	0.08498612	0.07866665	0.05321894
Ice7	Iceland	0.07386639	0.041409968	0.03937074	0.02562285	0.16616777	0.07689249	0.08861645	0.05794741
Ice8	Iceland	0.01435123	0.008763566	0.03157784	0.02260568	0.10915031	0.04174467	0.06753878	0.049389
Ice9	Iceland	0.03622774	0.013339926	0.03462808	0.02486291	0.06149489	0.03395084	0.07107993	0.05221892
Py1	Pyrenees	0.44832864	0.132843969	0.05670773	0.03694046	0.69952348	0.21850819	0.10055089	0.06357312
Py10	Pyrenees	0.21747876	0.077028975	0.05701202	0.03674902	0.42234954	0.14681252	0.09811096	0.06237338
Py2	Pyrenees	0.38081065	0.118947763	0.05558593	0.03666891	0.59951336	0.19433843	0.10342623	0.06576901
Py3	Pyrenees	0.06630627	0.027612382	0.05952395	0.03910609	0.14359257	0.0670625	0.11680764	0.07398462
Py4	Pyrenees	0.44832864	0.132843969	0.05670773	0.03694046	0.69952348	0.21850819	0.10055089	0.06357312
Py5	Pyrenees	0.44832864	0.132843969	0.05670773	0.03694046	0.69952348	0.21850819	0.10055089	0.06357312
Py6	Pyrenees	0.38081065	0.118947763	0.05558593	0.03666891	0.59951336	0.19433843	0.10342623	0.06576901
Py7	Pyrenees	0.1488375	0.061138559	0.05867021	0.03801489	0.29452979	0.12076896	0.11710907	0.07122017
Py8	Pyrenees	0.1488375	0.061138559	0.05867021	0.03801489	0.29452979	0.12076896	0.11710907	0.07122017
Py9	Pyrenees	0.04006381	0.01742079	0.05909746	0.03908731	0.126826	0.05843305	0.11286558	0.07206025
Al1	Alps	0.24071962	0.1578022	0.0659734	0.04130191	0.63043642	0.35809084	0.13080522	0.08025507
Al2	Alps	0.35012226	0.142662833	0.06254248	0.03980129	1.44793347	0.47518066	0.13111467	0.0806046
Al3	Alps	0.33944504	0.199858618	0.06368756	0.04067282	0.82903692	0.43965159	0.13403943	0.08177429
Al4	Alps	0.64003332	0.336894458	0.06695715	0.0416541	1.18249024	0.58733623	0.13451622	0.08116505
Al5	Alps	0.64003332	0.336894458	0.06695715	0.0416541	1.18249024	0.58733623	0.13451622	0.08116505
Al6	Alps	0.4340352	0.232159207	0.1144705	0.05943327	0.95161846	0.47873222	0.19893847	0.10297921
Al7	Alps	0.24071962	0.1578022	0.0659734	0.04130191	0.63043642	0.35809084	0.13080522	0.08025507
Al8	Alps	0.43605662	0.188170811	0.11436096	0.05940442	0.95579864	0.41746143	0.20420005	0.10522146
JHGO938	Sweden.M	0.03564232	0.016736385	0.03453756	0.03611316	0.13592777	0.0551895	0.05591991	0.06003043
JHGO939	Sweden.M	0.03564232	0.016736385	0.03453756	0.03611316	0.13592777	0.0551895	0.05591991	0.06003043
JHGO940	Sweden.M	0.03564232	0.016736385	0.03453756	0.03611316	0.13592777	0.0551895	0.05591991	0.06003043
JHGO941	Sweden.M	0.03564232	0.016736385	0.03453756	0.03611316	0.13592777	0.0551895	0.05591991	0.06003043
JHGO942	Sweden.M	0.03564232	0.016736385	0.03453756	0.03611316	0.13592777	0.0551895	0.05591991	0.06003043
Jam35	Sweden.M	0.07118741	0.030009491	0.02617064	0.02863815	0.12458331	0.05340604	0.04841393	0.05339899
P25065_11	Sweden.M	0.03034925	0.023623357	0.03484468	0.03479757	0.08076604	0.05724266	0.05612818	0.05851758
P25065_11	Sweden.M	0.05160991	0.034409492	0.0280157	0.02874622	0.11696228	0.07385809	0.04987319	0.05129914
Sva115	Svalbard	0.19142198	0.172631445	0.04608993	0.06265358	0.23573118	0.24537775	0.08298204	0.11402893
Sva126	Svalbard	0.19142198	0.172631445	0.04608993	0.06265358	0.23573118	0.24537775	0.08298204	0.11402893
Sva129	Svalbard	0.19142198	0.172631445	0.04608993	0.06265358	0.23573118	0.24537775	0.08298204	0.11402893
Sva188	Svalbard	0.19142198	0.172631445	0.04608993	0.06265358	0.23573118	0.24537775	0.08298204	0.11402893
Sva294	Svalbard	0.19142198	0.172631445	0.04608993	0.06265358	0.23573118	0.24537775	0.08298204	0.11402893
Sva296	Svalbard	0.19142198	0.172631445	0.04608993	0.06265358	0.23573118	0.24537775	0.08298204	0.11402893
Sva299	Svalbard	0.19142198	0.172631445	0.04608993	0.06265358	0.23573118	0.24537775	0.08298204	0.11402893
SvaG194	Svalbard	0.19142198	0.172631445	0.04608993	0.06265358	0.23573118	0.24537775	0.08298204	0.11402893

Table S9. Showing calculated offset values for all individuals after using the candidate SNP approach in both LEA and BayPass. For BayPass the calculations are based on 115,958 unique significantly associated SNPS for CHELSA and 104,706 SNPs for WorldClim. For LEA we used 37,781 unique significantly associated SNPS for CHELSA and 54,438 SNPs for WorldClim. These values are summarized in Figure S16.

Paper III [redacted]

Allele frequency fluctuations are associated with demographic cycling in Icelandic rock ptarmigan

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Abstract

Understanding how natural populations adapt to recurring environmental changes, such as demographic cycles, is a central question in evolutionary biology. Multi-annual population cycles impose fluctuating selective pressures, yet disentangling the resulting genomic signals from those of long-term directional selection and genetic drift has been challenging due to a lack of high-resolution temporal data. Here, we investigate the genomic consequences of demographic cycling in a population of rock ptarmigan (*Lagopus muta*) from northeastern Iceland. We analyzed whole-genome sequences from 91 individuals sampled over an 11-year period, encompassing two population peaks (in 2010 and 2018) and two troughs (in 2007 and 2013). Using a simulation-based framework, we identified 22,399 single-nucleotide polymorphisms (SNPs) exhibiting patterns of strong fluctuating selection. These patterns were highly correlated with fluctuating demography, consistent with density-dependent selection. The strongest fluctuating sites overlapped with numerous candidate genes that may be important in the context of density-depending selection. The most significant candidate, *CTNNA2* (Catenin Alpha-2), is associated with startle response and anxiety-like behaviors that may be associated with predator avoidance. We also found candidate genes such as *GUCY2C* and *TRAF2*, whose functions may intersect with key stages of host-parasite conflicts. Our findings suggest that multi-annual demographic cycles are a powerful evolutionary force driving rapid and widespread genomic changes, with density-dependent selection on traits potentially playing a key role in adaptation. This study highlights the significance of temporal genomics in clarifying the interplay between demography and natural selection in wild populations.

Introduction

Balancing selection encompasses various mechanisms of natural selection that may act to maintain multiple alleles at a given locus in a population. These forces can maintain or increase genetic diversity past the levels expected from drift alone (Charlesworth 2006). A major form of balancing selection is fluctuating selection in space and/or time (e.g., an allele with high fitness at low population densities, and low fitness at high population densities). Fluctuating selection can occur in short bursts periodically or consistently across evolutionary spans. However, interactions between demography and selection are currently poorly resolved (but see: Nespolo 2007, Gulisija and Kim 2015, Jain and Kaushik 2022). A key issue in detecting genomic patterns of fluctuating selection has been a lack of high-resolution longitudinal genomic data (Messer *et al*. 2016, Buffalo and Coop 2020). There has also been difficulty in differentiating between real signatures of selection and recurrent admixture/immigration events that may create an illusion of balancing or directional selection (Isildak *et al*. 2021). The extent to which populations may undergo directional selection in response to environmental shifts versus fluctuating selection to handle cyclical changes like seasonality can be unclear (Soni and Jensen 2024). Recent studies incorporating time-series genomic data have observed that seasonal disruption can maintain allele frequencies in systems such as *Drosophila* (Bergland *et al*. 2014, Machado *et al*. 2021, Rudman *et al*. 2022), and that allelic diversity trends can be reliably influenced by demography across phyla (Lynch *et al*. 2024).

In Iceland, the rock ptarmigan (*Lagopus muta*) provides an excellent opportunity to investigate trends in selection over time with influences from complex demography. Icelandic rock ptarmigan populations are well-studied with extensive historic sampling data available (Garðarsson 1971, 1988). There is good documentation of multi-annual demographic cycling in the present study population (Nielsen 1996, Sturludóttir *et al*. 2018, Johnson and Nielsen 2024) which exhibit clear cyclical shifts in phenotypic traits such as fat, gut length, and parasite loads (Stenkewitz *et al*. 2016; Guðmundsson 2015). This creates regularly shifting ecological conditions in which alleles at many loci may alternate between beneficial and deleterious depending on whether the population is in a peak or trough. For example, Eimeria parasite infections are common in Icelandic Rock Ptarmigan, affecting as much as 92% of the population (Skírnisson *et al.* 2012). High parasite intensity is negatively correlated with host condition, including body mass and fat reserves. Juveniles in autumn are the most vulnerable group, exhibiting the highest parasite loads and the lowest body condition (Stenkewitz *et al.* 2016). This parasite-driven health impact likely reduces winter survival and fitness, suggesting parasites play a tangible role in the ptarmigan's population cycling.

Using whole genome re-sequencing data from a single population of Icelandic rock ptarmigan across four different time points, we aimed to identify and quantify fluctuating allele frequency changes resulting from the population's regular demographic cycling. We attempt to disentangle genuine evidence of selection from drift and focus on detecting shifts coinciding with population density troughs and peaks over the course of an 11-year study period. We identify specific SNPs and genes under putative selection across time and speculate regarding the roles that these sites may play in population fitness and demography.

Methods:

Rock ptarmigan individuals were collected as part of the comprehensive "Rock ptarmigan health and population change" project to investigate the species' health status in Iceland between 2006 and 2018 (Icelandic Research Fund grant awarded to OKN, #090207021). We utilized a subset of 91 young-of-the-year birds from these studies collected from a 150.000 hectare area of northeastern Iceland located between Lake Mývatn and Húsavik to ensure capturing of local demographics (Fig. 1). Monitoring of Rock Ptarmigan takes place predominately in the spring and fall and includes transect-based surveys that are considered reliable (Johnson and Nielsen 2024, Ferrarini and Nielsen 2025). In the area where our study birds were sourced there are a handful of established census locations with some variability between exact timing of peak and trough demographics, but the northeastern region as a whole has seen spring census sizes fluctuating between 10,000 and 55,000 birds (Johnson and Nielsen 2024). Our collected individuals originated from two trough density years (2007 and 2013; n= 26 and 24) and two peak density years (2010 and 2018; n= 20 and 21; Fig. 2). Samples included both females and males at an approximately 1:1 ratio each year.

DNA extraction from muscle tissue was carried out using a Monarch™ Genomic DNA Purification Kit (New England BioLabs, USA). The extracted DNA underwent assessment for concentration and purity utilising both a NanoDrop® 2000 spectrophotometer and Qubit® 3.0 fluorometer Quantitation Kit (Invitrogen™). DNA Nanoball (DNB) general DNA library preparation and DNBSEQ PE150 paired-end whole genome sequencing (WGS) at approximately 20x coverage was performed by Beijing Genomics Institute Group (Hong Kong).

Read quality was determined using FastQC v.0.11.9 (Andrews 2010), while adapters and low quality sites were removed from the raw reads using Trimmomatic v.0.36 (Bolger *et al.* 2014). Subsequently, the trimmed reads were aligned to the rock ptarmigan reference genome (GenBank accession: GCA_023343835.1; Squires *et al.* 2023) using BWA-mem v.0.7.17 (Li and Durbin 2009) and bam files were sorted and marked for duplicates using Picard v.2.20.4

(http://broadinstitute.github.io/picard/). Evaluation of mapping quality and average read coverage was performed using Qualimap v.2.2.1 (García-Alcalde *et al.* 2012, Okonechnikov *et al.* 2016). Variant calling was conducted utilizing GATK v.4.1.1.0 (McKenna *et al.* 2010) following best practices (DePristo *et al.* 2011, Van der Auwera and O'Connor 2020). First, we employed Haplotypecaller with -ERC GVCF enabled on the duplicate-marked and down-sampled bam files. The resultant gVCFs were combined for each individual and chromosome using CombineGVCFs, and then jointly genotyped using GenotypeGVCFs. After calling all variant and invariant sites, Biallelic SNPs and INDELs were separately selected from the subsequent VCF with genotyped variants using SelectVariants. We applied "hard filtering" standards to filter the resulting VCFs for both SNPs and INDELs. For SNPs, this involved filtering out variants with Phred quality (QUAL) < 30.0, root mean square mapping quality (MQ) < 40.00, strand odds ratio (SOR) > 4.00, variant quality by depth (QD) < 2.00, fisher strand bias (FS) > 60.00, mapping quality rank sum test (MQRankSum) < -12.5, and read position rank sum test (ReadPosRankSym) < -8.00. For INDELs, filtering criteria included (QUAL) < 30.0, MQ < 40.00, SOR > 10.00, QD < 2.00, FS > 200.00, and ReadPosRankSum < -20.00. We then filtered the final VCF by read depth (10 < DP < 41), allowing a maximum of 10% missing data across samples using vcftools v.0.1.16 (Danecek *et al*. 2011). Indels were called and filtered by their own filtering criteria. We then intersected and removed any SNP within 5bp of an INDEL using BEDTools v.2.31.1 (Quinlan and Hall 2010). Next, vcftools was used to conduct a final minor allele frequency cutoff of minimum 0.05 across all samples resulting in 7,174,144 SNPs for investigation. To incorporate invariant sites into our vcf file, we re-executed GenotypeGVCFs in GATK v.4.1.1.0 (McKenna *et al.* 2010), specifying the option --include-non-variant-sites.

An initial analysis of relatedness using vcftools --relatedness and --relatedness2 functions showed no close relations among the tested individuals according to both A_{jk} statistics (Yang *et al.* 2010) and calculated relatedness Φ following the KING method (Manichaikul *et al.* 2010). Only one pair of individuals showed any potential relationship according to these methods (LM-13-122 and LM-18-052B; A_{jk}= 0.114, Φ= 0.099), although this was at a level below first-cousins.

Calculating Genetic Diversity (π), differentiation (F_{ST}), and allele frequencies.

The autosomal nucleotide diversity (π) and pairwise population differentiation (F_{ST}) were computed utilizing pixy v.1.2.5.beta1 (Korunes and Samuk 2021), which offers an unbiased estimation of nucleotide diversity by factoring invariant sites into its computation. The sliding window size was set to be 10k based on observed LD-decay calculated via plink v.1.90b4.9 (Purcell *et al.* 2007; Fig. S1). The mean π and F_{ST} values of each population (year) and comparison were visualised using ggnetwork v.0.5.13 (Briatte 2024).

To standardize F_{ST} values, we used z-transformation across all windows, enabling comparison across chromosomes and facilitating outlier detection. Windows exceeding the 95th percentile of z-transformed F_{ST} (ZF_{ST}) values were classified as outliers, representing the top 5% of differentiation signals. Gene annotations were obtained directly from NCBI (Assembly ID: GCF_023343835.1). Only autosomes were considered in the analysis resulting in 19,159 annotated genes. Windows with the top 10 ZF_{ST} values were matched to overlapping genes using tidyverse v2.0.0 (Wickham 2016; Wickham *et al*. 2019) in R v4.4.0 (R Core Team 2023) and RStudio v 2025.09.1+401 (RStudio Team 2020). Gene names and predicted functions were appended to the top windows by manually intersecting hit positions with the reference gene features file from NCBI (Fig. 3). Final visualizations across chromosomes 1–28 were generated using ggplot2 v3.4.2 and ggrepel v0.9.3 (Slowikowski 2024), with gene labels added for the top 10 annotated hits. Functional summaries were cross-referenced with GeneCards (https://www.genecards.org/) and NCBI for interpretive context.

The allele frequencies were calculated for each collection year for every SNP using VCFtools v.0.1.16 (Danecek *et al.* 2011). To monitor changes in allele frequency over time, we most often followed the frequencies of the minor alleles from 2007. We also verified whether a SNP was located within gene regions that overlap with significant ZFst windows.

Identification of Sites under Putative Density Dependent Selection

The magnitude of density dependent selection was estimated using a sum-of-deltas method whereby after filtering to consider only sites exhibiting fluctuating patterns (minor allele frequency change between time points: increasing → decreasing → increasing; decreasing → increasing → decreasing). We added the absolute value of allele frequency changes between each consecutive time point to assign each variant a total 'frequency change score' with the assumption that those exhibiting higher total scores showed greater frequency changes overall between years. Therefore, we expect the most strongly fluctuating sites to appear on top as they would be capable of generating the largest cumulative allele frequency changes (CAF):$|\Delta p_1| + |\Delta p_2| + |\Delta p_3|$= CAF, where Δp_1, Δp_2, and Δp_3 are the changes in allele frequency between 2007–2010, 2010–2013, and 2013–2018, respectively.

Importantly, this method is meant to capture fluctuation magnitude in a way that divergence calculations such as F_{ST} won't always find. The SNP positions in the top 5% of frequency change scores were then manually aligned (in R) with a gene features file from NCBI to identify those falling within annotated coding regions. A comparison was made to identify overlap between unique genes these SNPs could be associated with and between the genes associated with outlier ZF_{ST} windows that were previously identified. CAF was again calculated in later steps to identify the most strongly fluctuating sites among candidate SNPs.

Identifying loci under selection

To identify loci under fluctuating selection, we first identified candidate loci consisting of putatively selected SNPs at each time interval. We first estimated the contemporary variance effective population size (*Ne*) between each time interval from patterns of allele frequency change from the total set of 7,174,144 SNPs using the software varne (Gompert and Messina 2016; Rêgo *et al.* 2019). Estimates of contemporary *Ne* were subsequently used to parameterize null model of expected evolutionary change due to genetic drift. Due to our modest sample size at each time point, we also account for the effects of sampling error in our null models. To identify alleles under selection, we performed a three-step simulation procedure for each SNP at each time interval incorporating both sampling uncertainty and genetic drift. First, we accounted for sampling uncertainty at the initial time point (time 0) by inferring the posterior distribution of the underlying allele frequency (p_0) given the observed frequency, ($p_{0,obs}$) and number of genomes sampled (n_0, equal to twice the number of diploid individuals). Given an observed allele count $k_0 = p_{0,obs} \times n_0$, we drew 1,000 samples of the underlying frequency from $p_0 \sim beta(k_0+0.5, n_0-k_0+0.5)$. Second, for each of the 1,000 sampled underlying frequencies at time 0, we simulated the allele frequency at time 1 (p_1) under genetic drift using a Wright-Fisher model with a beta approximation (Gompert 2016), where $p_1|p_0 \sim beta(\alpha, \beta)$, with $\alpha = p_0 \frac{1-F}{F}$, and $\beta = (1-p_0)\frac{1-F}{F}$, where $F = 1 - \left(1 - \frac{1}{2N_e}\right)^t$, and t is the number of generations between samples. Third, we accounted for sampling uncertainty at the final time point by sampling what would be observed if we sampled n_1 gene copies from the simulated p_1 frequencies. For each simulated p_1, we drew $k_1 \sim Binomial(n_1, p_{1,})$ and calculated the final simulated frequency as $p_{1,sim} = k_1/n_1$. We calculated one-tailed p-values as the proportion of simulated allele frequency changes $\Delta p_{sim} = p_{1,sim-p_{0,obs}}$ that were as extreme or more extreme than the observed change $\Delta p_{obs} = p_{1,obs} - p_{0,obs}$ in the direction of the observed change. It should be noted that SNPs identified in this manner are not necessarily causal loci, but are likely in linkage disequilibrium with causal loci, and are representative of the patterns of evolutionary change in those loci.

To describe the overall allele frequency dynamics in putatively selected loci, we constructed clusters of SNPs based on correlations between allele frequency trajectories through time (Otte & Schlötterer 2021, Ament *et al.* 2023). We first filtered SNPs to retain only those with $p < 0.001$ in any time interval (accounting for drift and sampling error), including between 2007 and 2018, resulting in a set of 56,307 SNPs. Allele frequencies were normalized with an arcsine-square-root transformation followed by centering and scaling. We calculated Pearson correlations between the entire subset of SNPs, converted these to distances (1 - |r|), and performed hierarchical clustering using average linkage with a maximum distance threshold of 0.25. A total

of 6 clusters were observed with at least 10 SNPs in the cluster. Correlation of patterns to reported regional census data (sourced form Johnson & Nielsen 2024) was also noted.

We then calculated a second set of SNPs which are likely to be experiencing more explicit fluctuating selection. These fluctuating selection candidate SNPs were identified as those whose observed allele frequencies showed alternating directions of change and exhibited $p < 0.05$ across all time intervals, acting as a conservative threshold for fluctuating selection. Downstream investigation of candidate gene enrichment after identifying candidate sites utilized g:Profiler (Reimand *et al.* 2007).

Results

Diversity and Differentiation Among Sampling Years

Nucleotide diversity within each sampling year was around 3.18×10^{-3}, and the mean genome-wide F_{ST} values between each sampling year was expectedly low at 1.28×10^{-4} (Fig. S2). Genetic diversity appears to be influenced by the long-term effective population size beyond our cohorts rather than being impacted by short-term fluctuations in population size. These results are reasonable if one considers that the genetic diversity, π, is an estimator of the expected genetic diversity, θ, where $\theta = 4N_e\mu$, with μ representing the mutation rate, and the historical effective population size, N_e, of a constantly fluctuating population is calculated as the harmonic mean of its population size (Nielsen and Slatkin 2013). Hence, the genetic diversity of a population undergoing constant fluctuations is anticipated to remain stable as observed. As expected, genome wide differentiation among years was minimal (Fig. S2). The ZF_{ST} values among sliding windows varied between -2.075 and 21.605 for comparisons between trough and peak years, and between -2.021 and 9.721 for the years 2007 and 2018. Although there was a larger total range of values in peak/trough comparisons, the average value of the top 5% of windows was larger in the comparison between 2007 and 2018 than in the comparison between trough and peak years. We observed that in the 5% outlier windows, there were hits for 3602 genes in comparisons between 2007 and 2018 as opposed to the 3739 hits found in peak and trough assessments. Given 18,909 genes were available among autosomes, this implies that around 20% of all annotated genes were captured by our outlier window analysis. However, 10kb windows, particularly in gene-dense regions, contain many genes that are not themselves targets of selection but are linked to a causal variant (i.e., genetic hitchhiking). Importantly, even among the top ZF_{ST} windows between 2007 and 2018 many sites still showed clearly fluctuating patterns, with the most notable window in the top 10 being for the gene CTNNA2 (Fig. 4).

Temporal Allele Frequency Shifts.

Based on the patterns of allele frequency shifts, a total of 7,174,144 SNPs were classified into three groups: directional (showing consistent changes, n= 550,104), consistently fluctuating (exhibiting fluctuations consistent with peak and trough years; n=2,957,962), and inconsistently fluctuating (all others; n=3,666,078). We later detect which of these SNPs may be experiencing selection (see below). We found that 7.67% of the SNPs exhibited directional allele frequency changes from 2007 to 2018, 41.23% showed consistent fluctuating patterns, while the remainder accounted for 51.1% (Tab. 1). Using our CAF approach, we found that among the top 5% of most intensely consistently fluctuating sites, there were 97,315 SNPs (65.8%) that were within at least one gene region. Of the unique gene regions they were found in, they shared 80.33% of unique genes (total 1852 gene names) that were also identified in the top 5% of ZF_{ST} outlier windows (overlapping SNP hits shown in Fig. S3).

Subsequently, we examined the allele frequency shifts for windows exhibiting the 10 highest ZF_{ST} values in the comparison among trough and peak years (Fig. 3A). These windows consequently showed trends similar to the cycling population density (Fig. 4A). In the comparison between 2007 and 2018 (Fig. 3B), the allele frequency of all windows all showed an overall directional trend while several still showed evidence of fluctuating patterns (Fig. 4B).

Detecting putatively selected loci:

The effective population sizes associated with each time interval were large, with moderate variation (Ne_{07-10} = 2,878; Ne_{10-13} = 897; Ne_{13-18} = 5633; Ne_{07-18} = 1,261). This variation is unsurprising, as the census population size itself fluctuates, but importantly the relationship between drift and N_e is non-linear, such that there is more uncertainty around large estimates of N_e (Waples 2025). Despite this variation, *Ne* is sufficiently large between each time interval that we expect genetic drift to contribute relatively little to changes in allele frequency.

To summarize allele frequency dynamics across putatively selected loci, we clustered 53,053 SNPs ($p < 0.001$ in any time interval, including between 2007 and 2018) based on correlations in their temporal trajectories (Fig 5). Six major clusters were observed, each comprising at least 300 SNPs (393–11,752 SNPs). Each cluster contained SNPs occurring across at least 10 different chromosomes, indicating that linkage disequilibrium across the genome is quite prevalent. Clusters generally exhibited non-linear patterns of allele frequency change, exhibiting oscillating or stepwise patterns of change. Two clusters in particular (Fig 5A and 5D) exhibited allele frequency changes which were highly correlated with fluctuations in demography of the ptarmigan populations ($\overline{r_A} = 0.80 \pm 0.14$ SD; $\overline{r_D} = 0.86 \pm 0.11$ SD). A notable exception to the overall trend of non-linear allele frequency change occurs in one cluster (Fig 5C) which exhibits

monotonic changes on average, consistent with sustained directional selection over the course of sampling.

We then focus on 22,399 candidate SNPs exhibiting explicit fluctuating patterns ($p < 0.05$ at each sequential time interval with allele frequency changes in alternating directions; Fig. S4). Among these, the mean absolute allele frequency change between time points was 07–10: 18.6%; 10–13: 20.99%; and 13–18: 17.17%. CAF was recalculated for these 22,399 candidate SNPs, and annotated genes overlapping with top scoring sites were manually reviewed to compile a list of interest (Tab. S1). Among all candidates there were 15,196 SNPs that fell within a gene, and this included 2,761 unique names, some of which were predicted gene loci without formal description. Gene enrichment investigations were conducted using g:Profiler on the unique gene names overlapping with our 22,399 candidate SNPs (Tab. S2).

Discussion

The most often discussed mechanisms of balancing selection are heterozygote advantage, negative frequency-dependent selection, antagonistic selection among life stages, and forms and varying selection in space and time (Charlesworth 2006). These mechanisms have in common that they may all preserve genetic diversity within and among populations and thus it has been difficult to distinguish which may be most important in any given species. While most tests of balancing selection are designed to distinguish genomic patterns that are generated over moderate to long evolutionary time perspectives (Bitarello *et al*. 2023), we identify fluctuating selection acting at contemporary, rapid timescales that coincide with demographic cycling. However, this does not exclude the possibility that there may be genomic signals of long-term balancing selection in the studied ptarmigan genomes. Previous studies have suggested that interannual fluctuating selection may be a prevalent force influencing standing levels of variation in natural populations (Machado *et al*. 2021, Bitter *et al*. 2024). Multi-annual cycling can arise from a number of circumstances such as different life history stages (Durland *et al*. 2021, Arnqvist and Rowe 2022) and changing interannual conditions (Rudman *et al*. 2022,). Adaptive tracking has been suggested to influence both stress tolerance and reproductive output in *Drosophila* with minor allele frequencies evolving to rapidly address environmental change at the population level (Rudman *et al*. 2022). Other studies have evaluated multigenerational patterns of selection on a genome-wide scale in natural populations (Therkildsen *et al*. 2013, Bergland *et al*. 2014, Exposito-Alonso *et al*. 2019, Kelly 2022, Chen *et al*. 2019, Lynch *et al*. 2024). All invariably show that ecological conditions and thus the signals of selection can vary over short time periods.

Several grouse species are well known to show interannual fluctuations in density and the Icelandic Rock Ptarmigan is no exception (Garðarsson 1971, 1988, Nielsen 1996, Sturludóttir *et al*. 2018). Local population densities may differ as much as by a factor of 20 when comparing the lowest to highest abundances and often follow a cyclic pattern with a varying pattern of 4-10 years between peaks (Natural Science Institute of Iceland, 2025). In the case of the Icelandic rock ptarmigan, the cyclicity is likely related to density dependent predation by the principal predator the gyrfalcon (Nielsen and Pétursson 1995), but may possibly also be related to other factors such as Emeria parasite loads (Stenkewitz *et al*. 2016; Johnson and Nielsen 2024).

The patterns observed in allele frequency changes for rock ptarmigan suggest that there may be significant covariance across the genome. That is, we observed large clusters of SNPs on different chromosomes highly correlated in their temporal trajectories (Figure 5). A form of density-cued antagonistic pleiotropy may explain the observed allele frequency changes. We suggest that there are pleiotropic genes in rock ptarmigan that are initially beneficial under certain conditions but then become antagonistic as density changes. This might occur if ecological conditions change dramatically with changing population densities and traits may for example involve resource aggression and/or fear responses to predation risks. Some antagonistic pleiotropy may be preferentially maintained where one allele becomes more useful as the alternate becomes more harmful and then vice versa, scaling past neutrality. In cases of immune response to parasite loads however, simpler dynamics of negative frequency dependent selection may take over. Fluctuations in environment due to regular demographic cycling have likely been prevalent for thousands of years, possibly since Iceland was first colonized by rock ptarmigan approximately 10,000 years ago (Sahlman *et al.* 2009; Holder *et al.* 1999). It is therefore plausible that selection regimes fluctuating with density have contributed to the coevolution of allelic responses to these recurring pressures.

As shown by candidate SNP clustering (Fig. 5), demographic cycling appears to exert genome-wide effects. Two clusters exhibited correlations with census size of ≥ 0.8, each comprising of SNPs fluctuating across several chromosomes. Even among the top ZF_{ST} windows between 2007 and 2018 (Fig. 4), many sites still exhibited fluctuating patterns. These patterns also seem generally correlated with the regional census population sizes. This could mean that even while heavily fluctuating, some genomic regions are still undergoing long-term directional changes over time, but more data from preceding, intervening, and following years are needed to verify this.

While identifying specific ecological mechanisms under selection can be challenging, we note that many of our strongest identified CAF outlier genes among candidate SNPS are related to neurological functioning (*ERICH1*, *MCF2*, *LHFPL3*, *TMTC2*, *RBM45*, *FAIM2*, and most notably

CTNNA2). It may be that birds at different population densities require and select for different cognitive abilities (Hardie & Cooney 2023). Conditions at trough and peak years may vary extensively in terms of social interactions, predator detection, and other factors. Research on the sister species red grouse (*L. lagopus scotica/ L. scotica*) in Scotland has revealed that population density can drive differences in social structure (Piertney *et al*. 2008) as well as other factors such as predation and parasite transmission (Cattadori *et al*. 2005).

The gene region of Catenine Alpha-2 (*CTNNA2*) appeared on multiple occasions during our analysis, particularly in ZF_{st} outlier windows and candidate SNPs exhibiting strong fluctuations (Fig. 3, Fig 4., Tab. S1). A detailed examination of the traits associated with this large gene revealed that it is involved in brain development and may play a role in behavior (Schaffer *et al*., 2018). This gene is present across animal phyla and encodes αN-catenin. It has previously been associated with the control of startle responses for mice (*Mus musculus*; Park *et al.* 2022) and sustained anxiety (among other psychiatric disorders) in humans (Eszlari *et al.* 2021). Flushing behavior, naturally adjacent to startling, has been observed to reduce with increased population density among ptarmigan in Canada (Pelletier & Krebs 1998). This finding may corroborate the fluctuating allele frequency of *CTNNA2* with density in Icelandic Rock Ptarmigan as predation risk changes with population density.

The candidate genes we identified (Tab. S1) are diverse but may also possess interconnected roles that are critical for a ptarmigan's ability to survive an *Eimeria* infection. Notably we saw *GUCY2C* which is responsible for maintaining intestinal barrier integrity and handling toxicity in the gut (Bose *et al.* 2020; Lin *et al.* 2012). Considering that gut barrier integrity is compromised by *Eimeria*, a genus of monoxenous coccidian parasites that primarily infect the gastrointestinal tract of various vertebrate hosts (Madlala *et al.* 2021), these genes likely ensure the gut lining is structurally sound to resist the parasite's physical invasion. Another notable gene was *TRAF2* that modulates apoptosis and cell-survival programs in epithelial and immune cells, making it a plausible mediator of gut barrier and inflammatory responses (Etemadi *et al*. 2015; Dhillon *et al.* 2019). This is highly relevant to see fluctuating because *Eimeria* can trigger apoptosis and immune cascades in gut cells (Bremer *et al.* 2021). Many of the other genes we found are involved in cellular maintenance, molecular interactions, and immune response. Genetic variation within any of these functional groups could affect fitness in environments with high versus low parasite pressure in the ptarmigan population.

Although most clusters of candidate SNPs exhibited non-linear allele frequency changes (5 of 6 clusters), we did observe that 20% of candidates (n=10,947) exhibited monotonic directional change on average, consistent with constant directional selection. These sites could be linked to longer term environmental drivers affecting the Icelandic population across cohorts, potentially

related to climate change influencing the genome subtly across long time scales. We expect that a rapidly changing environment in the arctic may lead to more intense directional selection as the climate becomes increasingly warmer (Post *et al*. 2009, Box *et al*. 2019, Höglund *et al*. 2019). In the case of rock ptarmigan the most influential changes are expected to include increased seasonality of rainfall; decreased snow cover; and increasing temperatures (IPCC 2023). Each of these changes may have fitness effects that cause the genetic maintenance of extant minor alleles to be more or less adaptive/deleterious leading to extinction of alleles. Theoretically the more loci involved in maintenance of a single trait, the more vulnerable it becomes to loss as benefits decrease. Drift in very small populations for these useful but multi-loci traits could pose a problem for adaptive trait maintenance.

While an allele can sweep from a low frequency to fixation under differential selection, and balancing selection may cause regular shifting, the average prevalence of a biallelic site can nonetheless appear neutral unless allele frequencies are assessed at multiple time-points. Without temporal frequency data, evidence for fluctuating selection may be lost. Conducting further genomic investigations of juvenile birds from peak and trough cohorts collected prior to 2007 and after 2018 would improve our certainty in functionally important sites and more clearly distinguish fluctuation from directional trends. Although our 11-year time series captured genomic signals of generational turnover as population numbers and density shifted, adding population samples from older and more recent peak and trough years can substantially reduce false-positive assignments and increase confidence in sites inferred to be under directional or fluctuating selection. It is also important to note that specific manifestations of fluctuating selection may be localized and spatially dependent (Marostica *et al*. 2022); thus, it remains unclear whether these specific trends are shared across the entire island of Iceland.

Conclusion: We observed significant temporal fluctuation in allele frequency patterns coinciding with density changes in a cycling population of rock ptarmigan. We suggest that a portion of the identified allele frequency shifts are derived from short-term fluctuating balancing selection arising from the demographic cycling and establish that large portions of the genome show higher-than-expected patterns putatively attributable to shifting selection during peak and trough years across a significant number of genes. Our model suggests that loci responding to fluctuating pressures may be more common than those under consistent directional selection over this timescale. We hypothesize that candidate genes affect neurological development, behavior, and immune function, with parasite-mediated balancing selection conferring benefits in density-dependent environments.

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Ethical approval:

The Icelandic rock ptarmigan were collected specifically for a long-term study on the relationship between population changes and the ptarmigan's health that has been authorized by the Icelandic Institute of Natural History under law 64/1994, chapter 4, article 7 (http://www.althingi.is/lagas/140a/1994064.html)

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Tables and Figures:

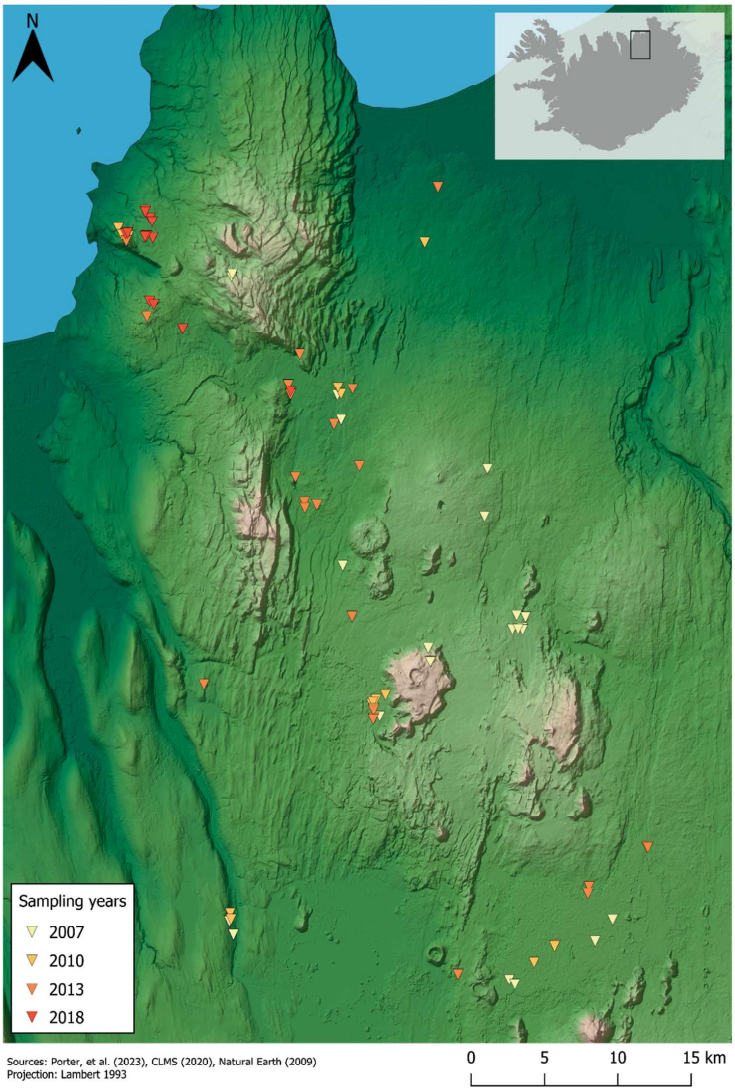

Figure 1. Relief map showing the years and locations of Rock Ptarmigan used in the present study from around northeastern Iceland approximately between Husavík and Reykjahlíð. Number of individuals from the various years are 2007, n = 26 (light yellow); 2010, n = 20 (orange); 2013, n = 24 (dark orange); and 2018, n = 21 (red).

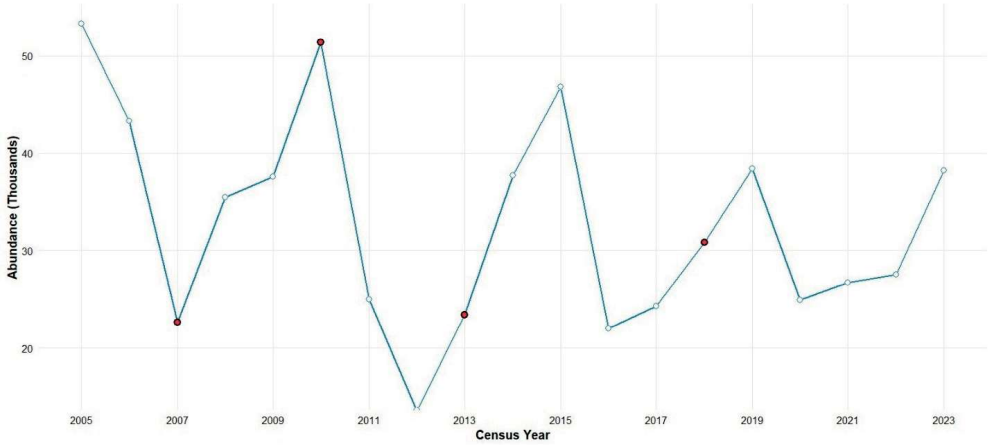

Figure 2. Overview of spring population census data from rock ptarmigan counted in the Northeastern Region (*Norðurland eystra*) of Iceland between 2005 and 2023 as collected by the Natural Science Institute of Iceland (methods detailed in Ferrarini and Nielsen 2025). Our sampling years are highlighted with bold red points. This figure was developed from data previously reported by Johnson and Nielsen (2024) and made available in their dryad repository (https://doi.org/10.5061/dryad.c59zw3rg3).

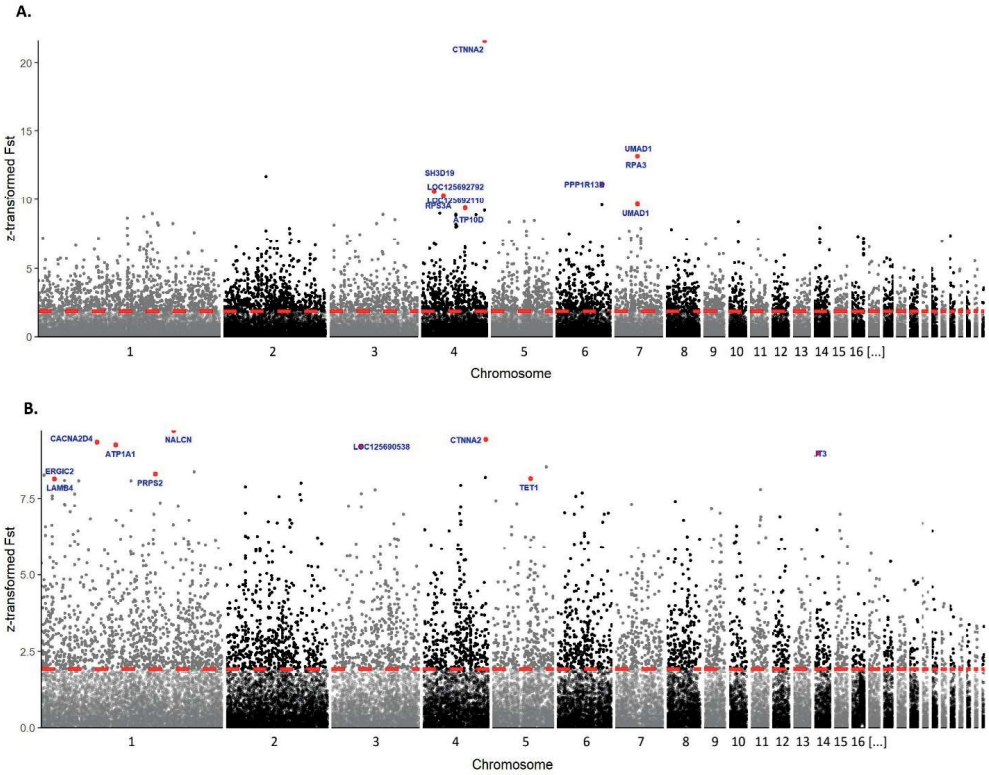

Figure 3. Differentiation (ZF_{ST}) among SNP:s in 10 kB sliding windows between A) peak (2010 and 2018) and trough years (2007 and 2013) and B) between the start (2007) and end (2018) year among the first 28 rock ptarmigan autosomes cut for visual clarity. Protein coding genes in the ten top outlier windows are indicated and the red line indicates the cutoff for 5% genome wide outliers (above the line).

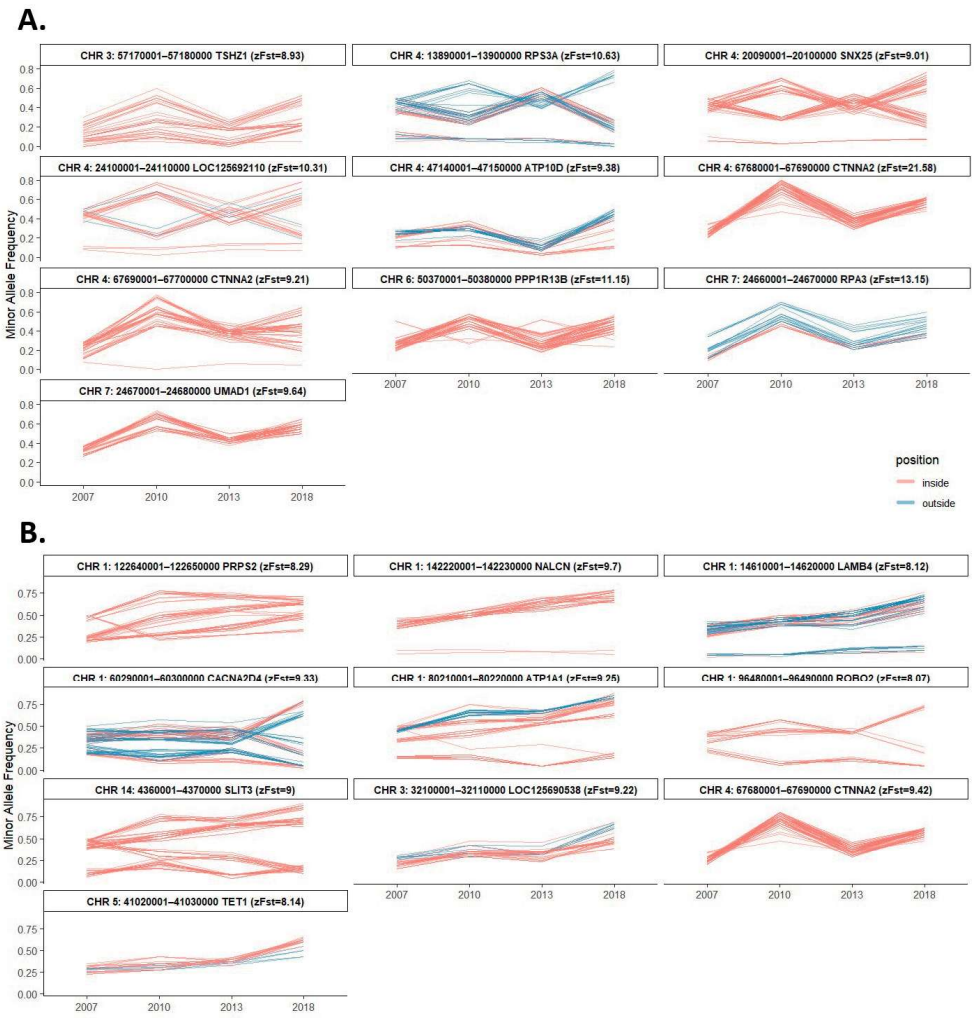

Figure 4. Temporal SNP frequency shifts of windows with the top 10 ZF_{ST} windows in different comparisons. A) Comparisons between trough years, i.e. 2007 and 2013, and peak years, 2010 and 2018. B) Comparisons between the earliest year, 2007, and the latest year, 2018. Each line represents a SNP and coloured by whether it is within the respective gene region. Overlap of these SNPs with top 5% of frequency change scoring sites is shown in Fig. S3. Fluctuating protein coding genes found in windows are indicated though some windows may include multiple other genes.

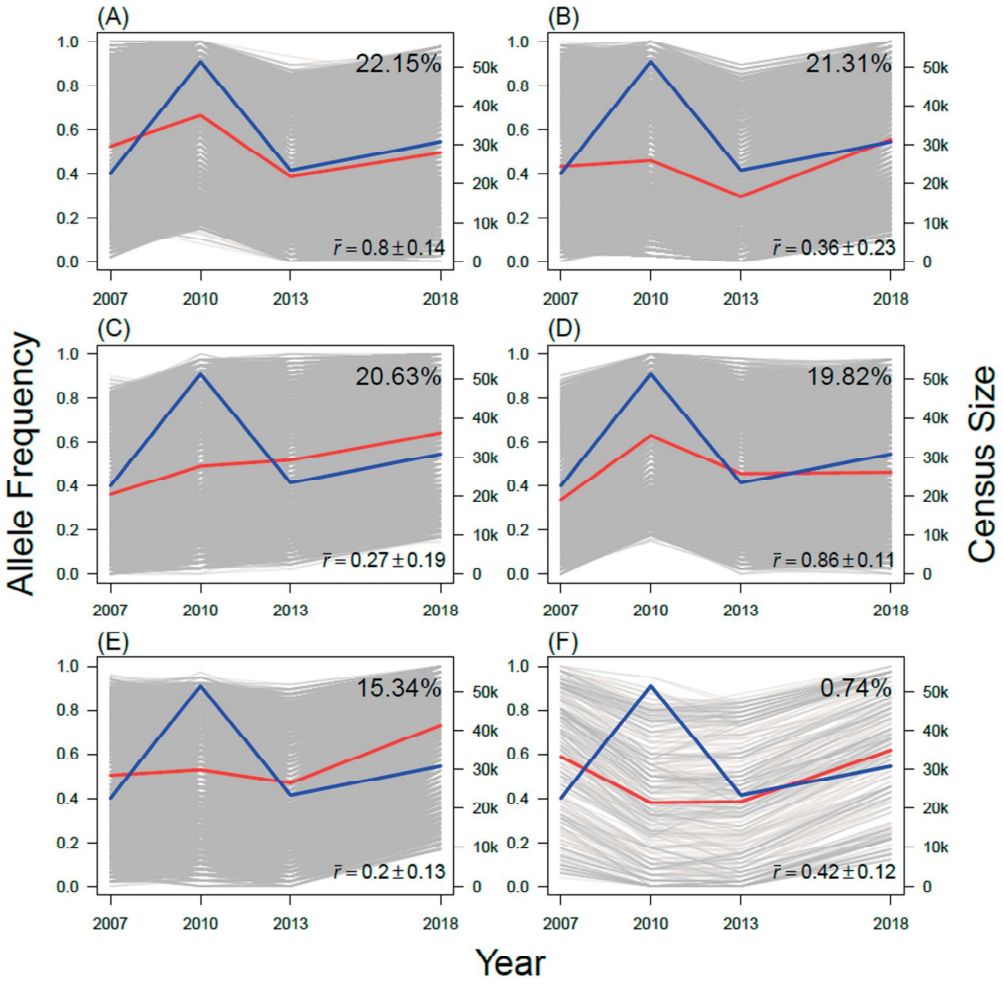

Figure 5. Clusters of allele frequency changes in putatively selected loci. Individual frequency trajectories are shown in grey, the mean allele frequency of the cluster is in red, and the census trend is shown in blue. The percentage of SNPs contained within each cluster is shown (of the 56,307 candidate SNPs which showed $p < 0.001$ in any time interval), as well as the mean absolute correlation and standard deviation between allele frequency and census size. Note that nearly all clusters exhibit patterns of fluctuating selection of some form (i.e., non-linear patterns of allele frequency change). Within each cluster, the allele shown (either reference or alternate) was chosen to ensure consistent directionality within the cluster. Regional census data as previously reported in Fig 2.

Category	2007-2010	2010-2013	2013-2018	Raw SNPs	Candidate SNPs
Directional	+	+	+	550,104	10,947
	-	-	-		
Consistently Fluctuating	+	-	+	2,957,962	22,399
	-	+	-		
Others	+	+	-	3,666,078	
	+	-	-		
	-	+	+		
	-	-	+		

Table 1. SNPs with different allele frequency shift patterns are grouped according to trend. **'**Directional' indicates that the allele frequency of the SNPs are increasing or decreasing consistently across years; The SNPs showing alternating increase and decrease in frequency are in the 'consistently fluctuating' group; The 'Others' group contains SNPs showing alternative patterns. Note that some sites may also show identical frequency between years and thus experience no change as opposed to an increase or decrease these are also marked as 'Others'. The 'Others' group can be considered as inconsistently fluctuating. Raw counts are provided along with reduced counts for candidate SNPs showing significant allele frequency change at each transition after accounting for drift and sampling error ($p < 0.05$).

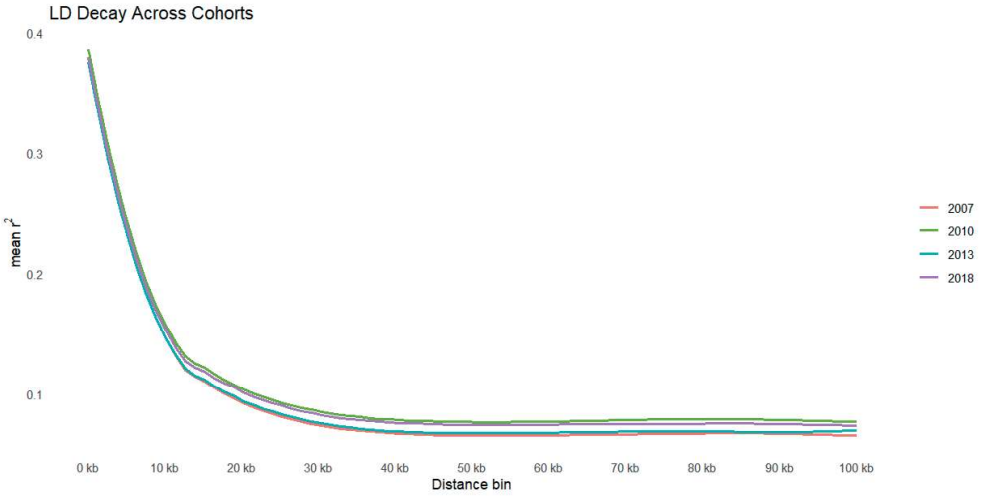

Figure S1. LD-decay among all SNPs as a function of physical distance showing a steep increase in average r^2 for sites with a genomic distance below 10kb. Trends are shown for each population cohort independently and largely align.

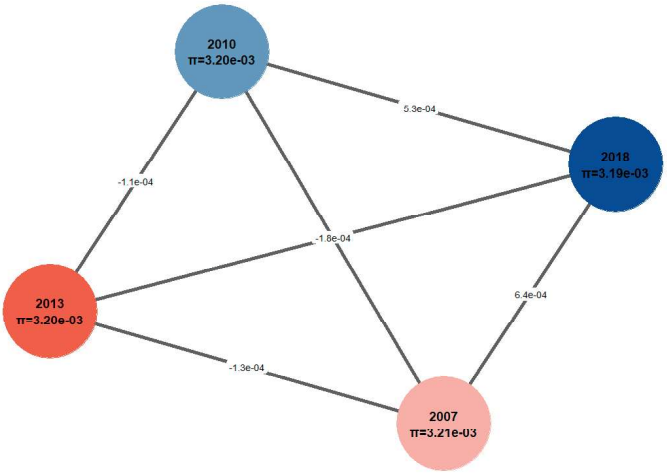

Figure S2. Genetic diversity (π) of each sampling year and the genetic differentiation (F_{ST}) between different groups. The circles represent collection years and the sizes indicate the π value, coloured by collection years. The values on each line represent the F_{ST} value between each two groups (2007, n = 26; 2010, n = 20; 2013, n = 24; 2018, n = 21).

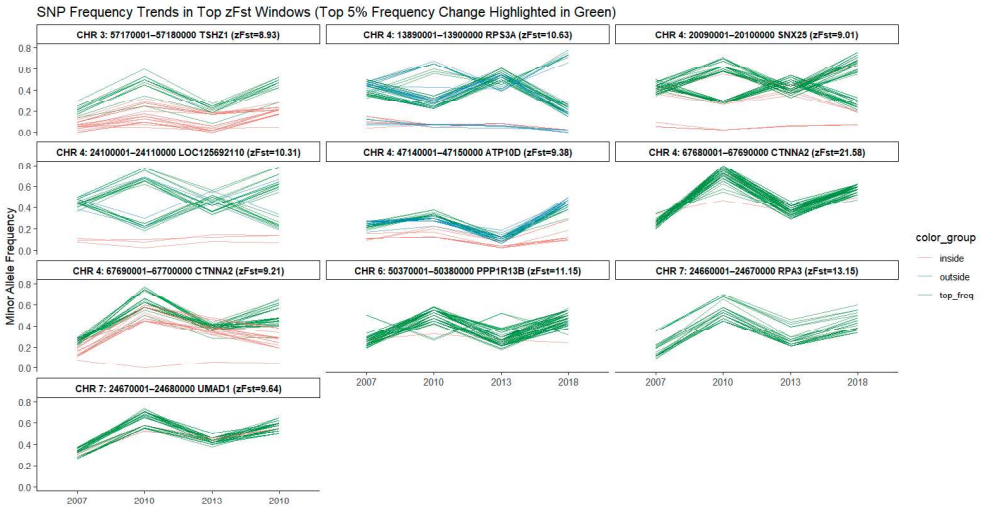

Figure S3. Temporal SNP frequency shifts of windows with the top 10 ZF_{ST} windows between trough years, i.e. 2007 and 2013, and peak years, 2010 and 2018. Each line represents a SNP and is coloured by whether it is within the respective gene region. Overlap of these SNPs with top 5% of frequency change scoring sites is shown in green. Here you can see that the SNPs picked up by the outlier CAF scores were also present in outlier ZF_{ST} windows.

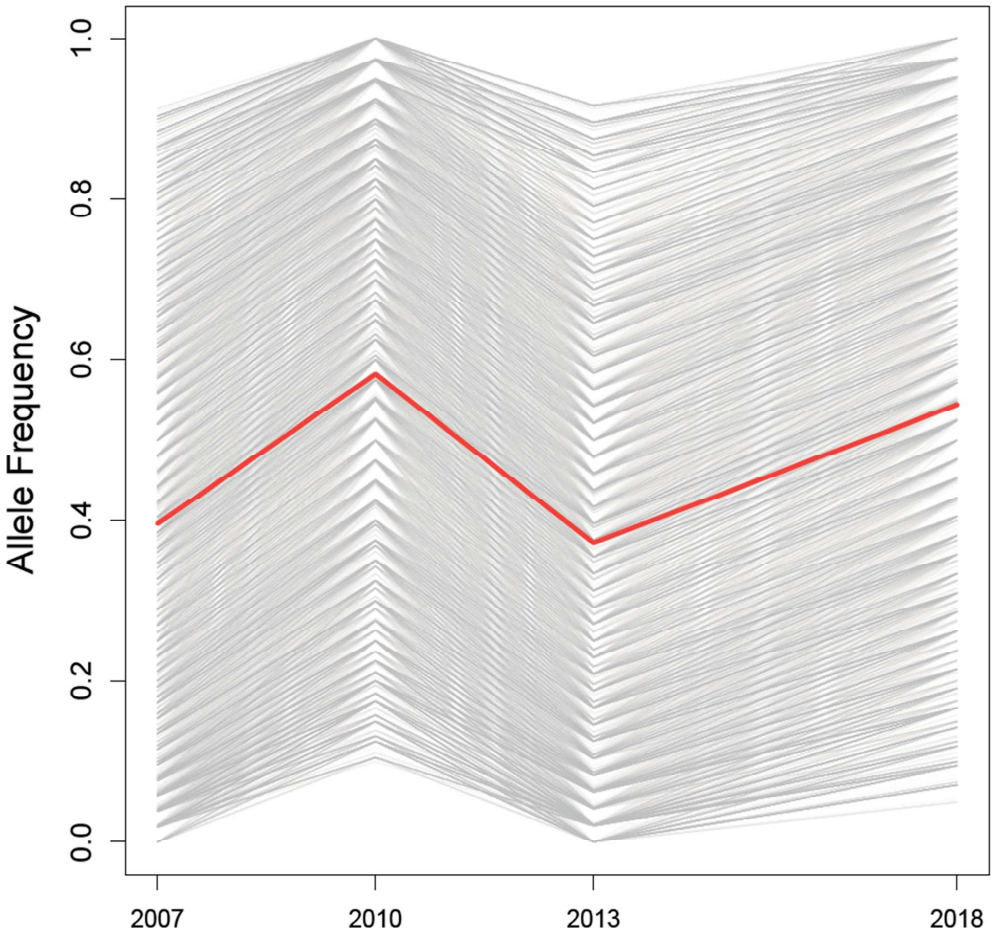

Figure S4. Allele frequency change of candidates SNPs showing significant ($p < 0.05$) changes across all three time points in explicitly fluctuating patterns (n=22,399). The average allele frequency change is indicated in red. The allele shown (either reference or alternate) was chosen to ensure consistent directionality within the cluster.

Chromosome	CAF	GeneCards Summary
4	1.1998	**CTNNA2** (Catenin Alpha 2) is a Protein Coding gene. Diseases associated with CTNNA2 include Cortical Dysplasia, Complex, With Other Brain Malformations 9 and Hereditary Breast Ovarian Cancer Syndrome. Among its related pathways are Blood-Brain Barrier and Immune Cell Transmigration: VCAM-1/CD106 Signaling and Sertoli-Sertoli Cell Junction Dynamics. Gene Ontology (GO) annotations related to this gene include structural molecule activity and structural constituent of cytoskeleton. An important paralog of this gene is CTNNA1.
2	1.1148	**ERICH1** (Glutamate Rich 1) is a Protein Coding gene. Diseases associated with ERICH1 include Ceroid Lipofuscinosis, Neuronal, 8.
2	1.0870	**HHIPL2** (HHIP Like 2) is a Protein Coding gene. Diseases associated with HHIPL2 include Lung Acinar Adenocarcinoma. Gene Ontology (GO) annotations related to this gene include quinone binding and oxidoreductase activity, acting on the CH-OH group of donors, quinone or similar compound as acceptor. An important paralog of this gene is HHIPL1.
7	1.0292	**UMAD1** (UBAP1-MVB12-Associated (UMA) Domain Containing 1) is a Protein Coding gene. Diseases associated with UMAD1 include Retinitis Pigmentosa.
1	1.0212	**GUCY2C** (Guanylate Cyclase 2C) is a Protein Coding gene. Diseases associated with GUCY2C include Diarrhea 6 and Meconium Ileus. Among its related pathways are Uptake and actions of bacterial toxins and Digestion and absorption. Gene Ontology (GO) annotations related to this gene include transferase activity, transferring phosphorus-containing groups and protein tyrosine kinase activity. An important paralog of this gene is GUCY2F.
1	1.0138	**RB1** (RB Transcriptional Corepressor 1) is a Protein Coding gene. Diseases associated with RB1 include Retinoblastoma and Familial Retinoblastoma. Among its related pathways are Aberrant regulation of mitotic G1/S transition in cancer due to RB1 defects and Cell-cell junction organization. Gene Ontology (GO) annotations related to this gene include DNA-binding transcription factor activity and enzyme binding. An important paralog of this gene is RBL2.
16	0.9951	**ERGIC3** (ERGIC And Golgi 3) is a Protein Coding gene. Diseases associated with ERGIC3 include Immunodeficiency 33 and Alpha Thalassemia-Intellectual Disability Syndrome Type 1. An important paralog of this gene is ERGIC2.
13	0.9905	**MCF2** (MCF.2 Cell Line Derived Transforming Sequence) is a Protein Coding gene. Diseases associated with MCF2 include Autism Spectrum Disorder and Skin Granular Cell Tumor. Among its related pathways are p75 NTR receptor-mediated signalling and Signaling by Rho GTPases. Gene Ontology (GO) annotations related to this gene include guanyl-nucleotide exchange factor activity. An important paralog of this gene is MCF2L.
8	0.9825	**ErbB4** (HER4) is one of the four members in the EGFR subfamily of receptor tyrosine kinases. Ligands include EGF, epiregulin, betacellulin and the neuregulins (Sundvall et. al.). Of these, NRG3 and NRG4 exclusively bind HER4 (Hynes et. al.). Mutations in ERBB4 have been identified in various cancer types including melanoma, lung adenocarcinoma and medulloblastoma. A therapeutic value of these aberrations still remains unknown (Arteaga et. al.).
1	0.9816	**ALG5** (ALG5 Dolichyl-Phosphate Beta-Glucosyltransferase) is a Protein Coding gene. Diseases associated with ALG5 include Polycystic Kidney Disease 7 and Autosomal Dominant Polycystic Kidney Disease. Among its related pathways are Synthesis of substrates in N-glycan biosythesis and Metabolism of proteins. Gene Ontology (GO) annotations related to this gene include oligosaccharyl transferase activity and dolichyl-phosphate beta-glucosyltransferase activity. An important paralog of this gene is DPM1.
1	0.9704	**PHLDA1** (Pleckstrin Homology Like Domain Family A Member 1) is a Protein Coding gene. Diseases associated with PHLDA1 include Malignant Syringoma and Fibroepithelial Basal Cell Carcinoma. Among its related pathways are Epigenetic regulation by WDR5-containing histone modifying complexes and Loss of proteins required for interphase microtubule organization from the centrosome. An important paralog of this gene is PHLDA3.
1	0.9690	**LHFPL3** (LHFPL Tetraspan Subfamily Member 3) is a Protein Coding gene. Diseases associated with LHFPL3 include Odonnell-Luria-Rodan Syndrome and Nasopharyngeal Carcinoma. An important paralog of this gene is LHFPL4.
8	0.9681	**CCNYL1** (Cyclin Y Like 1) is a Protein Coding gene. Gene Ontology (GO) annotations related to this gene include protein kinase binding. An important paralog of this gene is CCNY.
1	0.9661	**SCML2** (Scm Polycomb Group Protein Like 2) is a Protein Coding gene. Diseases associated with SCML2 include Nance-Horan Syndrome. Gene Ontology (GO) annotations related to this gene include DNA-binding transcription factor activity and sequence-specific DNA binding. An important paralog of this gene is SCMH1.
1	0.9595	**TMTC2** (Transmembrane O-Mannosyltransferase Targeting Cadherins 2) is a Protein Coding gene. Diseases associated with TMTC2 include Muscular Dystrophy-Dystroglycanopathy , Type C, 8 and Epilepsy, Familial Temporal Lobe, 5. Gene Ontology (GO) annotations related to this gene include identical protein binding. An important paralog of this gene is TMTC3.
8	0.9567	**RBM45** (RNA Binding Motif Protein 45) is a Protein Coding gene. Diseases associated with RBM45 include Dementia and Frontotemporal Dementia And/Or Amyotrophic Lateral Sclerosis 7. Gene Ontology (GO) annotations related to this gene include nucleic acid binding and RNA binding. An important paralog of this gene is PABPC1.
19	0.9525	**TRAF2** (TNF Receptor Associated Factor 2) is a Protein Coding gene. Diseases associated with TRAF2 include Myeloma, Multiple and Hematologic Cancer. Among its related pathways are Toll Like Receptor 7/8 (TLR7/8) Cascade and Apoptosis and survival TNFR1 signaling pathway. Gene Ontology (GO) annotations related to this gene include identical protein binding and signaling receptor binding. An important paralog of this gene is TRAF3.
1	0.9483	**AP1S2** (Adaptor Related Protein Complex 1 Subunit Sigma 2) is a Protein Coding gene. Diseases associated with AP1S2 include Pettigrew Syndrome and Basal Ganglia Calcification, Idiopathic, 6. Among its related pathways are Infectious disease and trans-Golgi Network Vesicle Budding. Gene Ontology (GO) annotations related to this gene include obsolete protein transporter activity. An important paralog of this gene is AP1S1.
1	0.9460	**PCDH9** (Protocadherin 9) is a Protein Coding gene. Diseases associated with PCDH9 include Autism Spectrum Disorder and Auditory Neuropathy, Autosomal Dominant 1. Gene Ontology (GO) annotations related to this gene include calcium ion binding. An important paralog of this gene is PCDH11X.
28	0.9410	**FAIM2** (Fas Apoptotic Inhibitory Molecule 2) is a Protein Coding gene. Diseases associated with FAIM2 include Obsessive-Compulsive Disorder. Among its related pathways is TRAIL signaling pathway. An important paralog of this gene is TMBIM1.

Table S1. Top twenty genes and corresponding gene card information for candidate sites after accounting for strongly fluctuating variants as calculated by CAF.

source	term_name	term_id	highlighted	adjusted_p_value	negative_log10_of_adjusted_p_value	term_size	query_size	intersection_size	effective_domain_size
GO:BP	anatomical structure development	GO:0048856	true	5,28016334129154e-21	20,032444379643	3455	1780	654	12952
GO:CC	cell junction	GO:0030054	true	1,39787304086749e-20	19,854532270760927	1320	1886	299	14220
TF	Factor: Msx-2, motif: NNNGACYAATTAGYNNT	TF:M01393	false	1,44834719332057 1e-20	19,839127317925 42	7266	2128	1121	17007
GO:BP	developmental process	GO:0032502	false	2,22652408194383 4e-20	19,652372603205 67	3691	1780	688	12952
TF	Factor: HOXA13, motif: ATAAMA	TF:M01292	false	5,99813376096768 7e-17	16,221983853514402	10953	2128	1553	17007
GO:BP	multicellular organism development	GO:0007275	false	6,79791832543574 1e-17	16,167624057614926	2779	1780	534	12952
TF	Factor: Cdx-1, motif: TTTATK, match class: 1	TF:M02086_1	false	1,02336977227984 5e-16	15,989967415120342	7590	2128	1142	17007
GO:CC	synapse	GO:0045202	false	6,91442413716279 1e-16	15,16022439839 5188	923	1886	216	14220
TF	Factor: Msx-1, motif: WNGNAATTANV	TF:M08822	false	1,74003345488586 34e-15	14,759442401641166	5705	2128	892	17007
TF	Factor: FOXD3, motif: NAWTGTTTRTTT	TF:M00130	false	2,85598079894552 1e-15	14,544244716692795	3399	2128	578	17007
GO:BP	system development	GO:0048731	false	1,10058433929042 4e-14	13,958376671252411	2360	1780	459	12952
GO:MF	protein binding	GO:0005515	true	1,54856595828751 e-14	13,810070289832957	4602	1675	782	12303
TF	Factor: GATA-3, motif: AGATAA	TF:M01878	false	5,55216246834994 e-14	13,449507178734754	8625	2128	1257	17007
TF	Factor: Foxc1, motif: TTVYTTTNW	TF:M07254	false	5,89442348072417 e-14	13,244550235840862	7233	2128	1081	17007
TF	Factor: HOXA13, motif: ATAAMA, match class: 1	TF:M01292_1	false	1,25240348125434 63e-13	12,902255573386297	6570	2128	994	17007
GO:CC	cytoplasm	GO:0005737	false	1,86393936092947 25e-13	12,778862504882527	7378	1886	1140	14220
TF	Factor: Lhx2, motif: WATTAN	TF:M07047	false	9,97956538633567 81e-13	12,000883871980867	6597	2128	992	17007
GO:BP	nervous system development	GO:0007399	false	2,00869761962104 2e-12	11,697085435085421	1421	1780	298	12952
TF	Factor: Cdx-1, motif: TTTATK	TF:M02086	false	4,74926656934426 45e-12	11,323373453423622	11889	2128	1636	17007
GO:CC	cell periphery	GO:0071944	false	7,99037911914339 1e-12	11,097432814247 23	3420	1886	587	14220
TF	Factor: Foxc1, motif: TTVYTTTNW, match class: 1	TF:M07254_1	false	1,26526800292402 57e-11	10,897817474596184	2749	2128	468	17007
TF	Factor: Oct-1, motif: NNNRTAATNANNN	TF:M00137	false	1,45631209033214 3e-11	10,836745544950915	6097	2128	920	17007
TF	Factor: Msx-2, motif: NNNGACYAATTAGYNNT, match class: 1	TF:M01393_1	false	2,06482273877367 97e-11	10,685117225788114	3421	2128	561	17007
TF	Factor: CdxA, motif: AWTWMTR	TF:M00101	false	2,37515338348698 e-11	10,624308339027925	5041	2128	780	17007
GO:BP	multicellular organism process	GO:0032501	false	3,18477668851376 3e-11	10,496920986754 94	4134	1780	709	12952
GO:CC	glutamatergic synapse	GO:0098978	false	3,40116117207710 6e-11	10,48837278750708	335	1886	95	14220
TF	Factor: Msx-2, motif: TAATTAN	TF:M07425	false	4,97041670938537 04e-11	10,303607198395412	3698	2128	597	17007
TF	Factor: Octamer, motif: TNATTTGCATN	TF:M00795	false	5,04462651777749 5e-11	10,29717098151 2801	4205	2128	666	17007
TF	Factor: GATA-3, motif: AGATAA, match class: 1	TF:M01878_1	false	3,98208101182524 79e-11	10,157260927105 27	3624	2128	586	17007
GO:CC	cell projection	GO:0042995	false	7,26788842716853 2e-11	10,1507103148 3144	1265	1886	296	14220
TF	Factor: Pax-5, motif: NNNNTTCACGCWTGANTKNNN	TF:M00097	false	7,07903250743901 4e-11	10,114993493920145	9452	2128	1338	17007
TF	Factor: FOX, motif: KWTTGTTTRTTTW	TF:M00609	false	1,32809644805757 527e-10	9,8767801044371466	3115	2128	514	17007
GO:BP	cellular developmental process	GO:0048869	false	1,77442534274534 74e-10	9,7509422694586292	2434	1780	451	12952
GO:BP	cell differentiation	GO:0030154	false	1,77442534274534 74e-10	9,7509422694586292	2434	1780	451	12952
TF	Factor: Msx-1, motif: CNGTAWNTG	TF:M00394	false	4,15359218328607 6e-10	9,3815761465196 99	7967	2128	1149	17007
TF	Factor: Msx-1, motif: WNGNAATTANV, match class: 1	TF:M08822_1	false	4,72579710098090 12e-10	9,3255254682157 5	1921	2128	341	17007
GO:CC	synaptic membrane	GO:0097060	false	5,52826846908263e-10	9,2574108722 71858	230	1886	71	14220
GO:CC	plasma membrane	GO:0005886	false	5,57919707767467e-10	9,181827104260256	3149	1886	537	14220
GO:BP	cell junction organization	GO:0034330	false	7,76259029474147 1e-10	9,109993350562 79	458	1780	120	12952
GO:MF	binding	GO:0005488	false	3,57022554711349 7e-10	9,0670077483711 04	9182	1675	1362	12303
TF	Factor: HNF3beta, motif: KGNANTRTTTRYTTW	TF:M00131	false	9,01292551656804 74e-10	9,0451342179573 49	2269	2128	390	17007
TF	Factor: GATA-4, motif: AGATAAN	TF:M03549	false	1,14037595060 9669e-9	8,942951950094 95	4052	2128	637	17007
GO:BP	positive regulation of cellular process	GO:0048522	false	1,22299862439370 383e-9	8,915754876765778	3506	1780	608	12952
GO:BP	localization	GO:0051179	true	3,36124112709582 86e-9	8,473500331204761	3503	1780	605	12952
GO:CC	plasma membrane region	GO:0098590	false	4,53677480651651 45e-9	8,343252777426615	691	1886	154	14220
GO:CC	plasma membrane bounded cell projection	GO:0120025	false	4,71865909254872 6e-9	8,326181397857944	1206	1886	239	14220
GO:BP	regulation of cell communication	GO:0010646	false	4,93074119689307 4e-9	8,307087791975231	2081	1780	389	12952
GO:CC	cytosol	GO:0005829	false	1,05783734196455 67e-8	7,975581106325437	2445	1886	427	14220
GO:BP	regulation of signaling	GO:0023051	false	1,13875495957838 04e-8	7,94433314233 8	2080	1780	387	12952
GO:BP	cell development	GO:0048468	false	1,26145947949566 46e-8	7,899126695296285	1637	1780	317	12952

Table S2. Top fifty gene terms and corresponding information from a g:Profiler enrichment analysis on genes encompassed by all 22,399 candidate SNPs .

Paper IV [redacted]

Genome-Wide Association study suggests highly polygenic architecture for condition-related traits in Icelandic Rock ptarmigan (*Lagopus muta*)

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Abstract:

Genome-Wide Association Studies (GWAS) are increasingly powerful tools for dissecting the genetic basis of complex traits in non-model organisms, providing crucial insights for ecology, evolution, and conservation. The Icelandic rock ptarmigan (*Lagopus muta*) offers a unique system for such research, as its populations exhibit well-documented, multi-year demographic cycles that mirror fluctuations in individual phenotypic condition. Here, we present the first exploratory GWAS for this population, leveraging whole-genome resequencing (WGS) data (20x coverage) from 90 young-of-the-year birds sampled across four years, representing two demographic "peak" and two "trough" years. We investigated the genetic architecture of morphological and condition-related traits, including body size metrics (Wing, Head, Sternum) and energetic reserves (Total Fat, Lean Dry Body Mass). Phenotypic analysis confirmed that Total Fat fluctuated significantly with population cycle position, independent of sex. Using a univariate linear mixed model in GEMMA, we tested for associations between 6,024,856 high-quality SNPs and our target phenotypes, including a PCA-derived 'size' trait, Total Fat, and Lean Dry Body Mass. We found no genome-wide significant associations (FDR < 10%) for any trait. This null result may suggest that these complex traits are highly polygenic, with no single locus of large effect. A follow-up GxE analysis testing for SNP-by-cycle-phase interactions, and a targeted analysis using *a priori* SNPs displaying high multi-annual fluctuation, also failed to yield significant hits from reduced tests on 133,930 SNPs. While non-significant, we identify a set of putative candidate genes (e.g., *HIVEP3, LAMA3, HTR1F*) from the analyses with the strongest associations to provide testable hypotheses for future investigations. This study provides an important foundational genomic resource and underscores the polygenic nature of complex traits in this ecologically significant, non-model species.

Introduction:

Genome wide association studies (GWAS) have become widely used in research over the last two decades, and use has gradually shifted from the investigation of human diseases towards applications in ecological genetics and conservation. In essence GWAS uses correlative statistics to determine which variants might be associated with diseases, complex traits, or discrete phenotypes (Wang *et al.* 2005; Tam *et al.* 2019; Uffelmann *et al.* 2021). These studies can take place at different scales, but most frequently investigate thousands of individuals across a population and often employ sequence reads from hundreds of thousands of comparable sites (Tam *et al.* 2019; Uffelmann *et al.,* 2021).

SNP associations can help researchers to identify the causative agents behind observed variation. Despite historical concerns about the applicability of GWAS to non-model and resource poor animal models, important trait loci have been uncovered using these methods. A notable example comes from the discovery of a haplotype for horn shape on chromosome 10 in Soay Sheep from the Atlantic island of St. Kilda (Johnston *et al.* 2011; McCairns and Merilä 2011). Structural variants in Eurasian Blackcap have been qualified using GWAS to explain migration patterns (Delmore *et al.* 2023) and GWAS identified that resistance to a parasitic gapeworm (*Syngamus trachea*) in House Sparrows (*Passer domesticus*) was associated with polymorphisms in immune genes (Lundregan *et al.* 2020). In Atlantic Cod (*Gadus morhua*), GWAS has just been used to provide evidence of fisheries induced evolution on populations in the Baltic Sea with derivative bespoke tests indicating that polygenic traits and multiple sites of moderate effect size may be responsible for declining fish sizes (Han *et al.* 2025). Together these examples show that GWAS has the potential to explain the genetics underlying both disease response and important life history traits in natural systems.

In Iceland, the rock ptarmigan (*Lagopus muta*) is a game bird of cultural importance that has been studied for almost a century. Variations in population size, fecundity, and age-specific survival rates are already well understood for Icelandic ptarmigan (Nielsen & Pétursson 1995; Brynjarsdóttir *et al.* 2003; Sturludóttir *et al.* 2018), and multiannual demographic cycling is recognized to occur with peak and trough densities five-to-ten years apart (Garðarsson 1988, Nielsen & Pétursson 1995, Magnússon 2005, Guðmundsson 2015). There is currently no comprehensive understanding of what drives population cycling in ptarmigan, but the phenomenon is known in many species (Moss and Watson 2001, Berryman 2002). Though a portion of survival is established to be strongly correlated with predation from Gyrfalcon (*Falco rusticolus*; Nielsen & Pétursson 1995, Brynjarsdóttir *et al.* 2003, Nielsen 2011, Barraquand & Nielsen 2018), cycling could also be influenced by mechanisms such as density effects influencing territoriality (see Matthiopoulous *et al.* 1998), plant-herbivore interactions (Garðarsson 1971, Dépré & Nielsen 2023), pathogen/parasite loads (Stenkewitz *et al.* 2016), and genetics (Chitty 1977, Henderson 1977).

These fluctuations seem to cascade into several health and fitness traits that are likely to effect individual survival. After long-term population declines were deemed to be perilous in the early years of the 21st century, hunting rules were changed in 2005 and a ptarmigan health initiative was developed (Sturludóttir *et al.* 2018; Nielsen *et al.* 2012; Nielsen *et al.* 2014). Data and tissue samples have been collected from 1294 birds in the Northeast Region of Iceland during the first week of October each year from 2006 to 2018. Notably, this data includes valuable response metrics potentially attributable to genetic factors such as body size measurements, tissue and organ weights, parasite loads, and crop contents (Nielsen *et al.* 2012; Nielsen *et al.* 2014; Dépré & Nielsen 2023). With the publication of a new reference genome for the Icelandic rock

ptarmigan (Squires *et al.* 2023) ultimately this data creates an opportunity for fine-scale investigations of the genetics behind phenotypic responses to interannual changes.

It is important to note that in some non-model systems with limited genomic resources, small sets of individuals have been utilized to perform GWAS (Han *et al.* 2025). Limitations around these approaches usually result from a reduced statistical power of detection and uncertainty around polygenic alleles and capturing small-effect loci. However, even with conventionally adequate sample sizes GWAS may have considerable limitations in identifying trait-associated variants, as correlative patterns might break down across landscapes at larger scales. Such limitations of predictive power have been previously shown in genetic studies of parasite loads in the closely related Red Grouse (*Lagopus scotica*; Wenzel *et al.* 2015).

With this in mind, the present study investigates the role that genetics may play in multi-annual phenotypic variability observed in Icelandic rock ptarmigan. We perform here an empirical investigation of whole genome resequencing data from 90 individual birds to identify specific SNPs or haplotypes that may be associated with observed phenotypic variability in a population exhibiting strong demographic cycling. We explore the phenotypic trends in available data, seek associations of traits with SNPs, and suggest regions of interest for further study.

Methods:

Collection and measurements:

A total of 1294 rock ptarmigan were collected by hunters in the autumn of 2006 to 2018 under a license issued by the Natural Science Institute of Iceland. The study area was in northeast Iceland, with most birds collected in upland regions east and north of Lake Mývatn (65° 37′ N, 17° 00′ W). The purpose of this study, referred to as the "rock ptarmigan health study", was to examine the relationship between health-related parameters and population change.

Each bird was tagged immediately after collection, then wrapped in absorbent paper, placed in a closed paper bag, cooled to 4°C, transported to the laboratory, and dissected within three days. Birds were sexed using both the loral stripe and the size and color of the combs (Montgomerie & Holder 2020), and aged based on the pigmentation of the primaries (Weeden & Watson 1967). Sex and age were verified during dissection by examining the gonads and the presence or absence of the bursa of Fabricius (Bursa fabricii). Two age categories were identified: juvenile birds (approximately 3 months old) and adult birds (15 months and older).

To establish an index of structural body size, we recorded the following external and internal morphometrics for each bird: (1) wing length, measured to the nearest millimeter with a ruler from the carpal joint to the tip of the flat, straightened wing; (2) head + bill length, measured to

the nearest 0.1 mm with calipers from the hindmost point of the head to the tip of the bill; (3) sternum length, measured to the nearest millimeter with calipers from the tip of the spina externa along the centerline to the margo caudalis; and (4) sternum–coracoid length, measured to the nearest 0.1 mm with calipers from the centerline of the margo caudalis to the cranial end of the coracoideum. Anatomical terms follow Baumel (1979).

We used lean dry body mass (hereafter, LDBM) and total body fat (hereafter, TF) as our condition indicators. Both LDBM and TF were calculated using functions derived from a whole carcass analysis conducted on 58 individual ptarmigan collected in our study area on October 5, as well as October 20 and 21, 2006, specifically for this purpose (unpublished data). Each carcass was divided into 12 parts. The different parts were oven-dried at 55°C (Memmert UFE-800 universal oven). After drying, the individual parts were weighed, packed in filter paper (Bravilor Bonamat B20, 203/535), and then washed in petroleum ether (boiling point 40–60°C) in a Soxhlet extractor to remove fat (for details, see Piersma *et al.* 1999). Once fat was no longer recoverable, samples were removed from the Soxhlet and dried at 55°C to calculate the fat-free dry mass. For the 1294 rock ptarmigan collected for the health project, we dissected several body parts and tissues, treating them the same way as described above.

In 2007, we used the following function to calculate LDBM:

$$LDBM = 17.4934 + PmajFFDM \times 3.3655$$

The function used in 2010, 2013, and 2018 to calculate LDBM was:

$$LDBM = 15.9110 + PmajFFDM \times 1.8910 + LegFFDM \times 3.3067$$

In 2007, we used the following function to calculate TF:

$$TF = 1.7115 + PminF \times 8.251 + HF \times 16.710$$

The function used from 2010, 2013, and 2018 to calculate TF was:

$$TF = 0.5824 + PminF \times 7.321 + LegF \times 4.112 + HF \times 6.379$$

Where PmajFFDM is the lean mass of the pectoralis major muscles, LegFFDM is the lean mass of the legs, FPmin is the fat content of the pectoralis minor muscles, FL is the fat content of the legs, and FH is the fat content of the heart. All mass values are in grams.

We had access to tissue samples for DNA extraction from all the analyzed birds (n = 1294); out of these, we selected 98 birds for our genomic investigations. After accounting for closely related individuals and demultiplexing contamination, our subset consisted of 90 juveniles originating from two trough years (2007 and 2013; n = 25 and 24) and two peak years (2010 and 2018; n = 20 and 21), respectively (Fig. 1). The birds included an approximately even sex ratio of 42 females and 48 males.

Phenotype data were investigated independently of genetic material for associations with population cycle position (collection year) and sex. Pairs.panel assessment from the R package 'psych' v.2.4.6.26 (Revelle 2024) and Python's 'seaborn' v0.13 (Waskom 2021) allowed for comprehensive assessment of terms before moving forward with genetic data.

Ptarmigan density surveys:

Territorial male ptarmigan were surveyed across six plots each spring, starting in 1981. The total area of these plots was 26.8 km² (range 2.4–8.0 km²). Each plot was surveyed once in May. The survey was conducted on foot by at least two observers during the early morning hours (05:00–10:00) or late afternoon (16:00–23:00). The locations of territorial males and ptarmigan kills were plotted on a map. A "kill" refers to the remains of a ptarmigan that was killed and eaten after arriving on the census plot in spring. The leading cause of death was predation by the Gyrfalcon (84%) (Nielsen, 1986). The "freshness" of the kill was based on the state of the feathers. The total number of males in spring is the sum of the number of territorial males censused and killed. Not all kills could be sexed, so to estimate the proportion of males, we used the sex ratio of ptarmigan killed by gyrfalcons in spring in the study area (73% males, see Nielsen, Brynjarsdóttir & Magnússon. 2004). Nielsen (1995) provides a detailed description of the census plots and methods. The ptarmigan population abundance index used was the annual mean density of males on these six plots, covering the years 2006–2018.

Sequence data and variant calling:

DNA extraction from muscle tissue was carried out using a Monarch™ Genomic DNA Purification Kits (New England BioLabs, USA). The extracted DNA underwent assessment for concentration and purity utilising both a NanoDrop® 2000 spectrophotometer and Qubit® 3.0 fluorometer Quantitation Kit (Invitrogen™). DNA Nanoball (DNB) general DNA library preparation and DNBSEQ PE150 paired-end whole genome sequencing (WGS) at approximately 20x coverage was performed by Beijing Genomics Institute Group (Hong Kong).

Bioinformatic analyses were initiated by removing adapters from the raw reads using Trimmomatic v.0.36 (Bolger *et al.* 2014) and then conducting an assessment of read quality using FastQC v.0.11.9 (Andrews 2010). Subsequently, the trimmed reads were mapped onto the rock ptarmigan reference genome (GenBank accession: GCA_023343835.1; Squires *et al.* 2023) using BWA-mem v.0.7.17 (Li & Durbin 2009). Evaluation of mapping quality and average read coverage was performed using Qualimap v.2.2.1 (García-Alcalde *et al.* 2012, Okonechnikov *et al.* 2016). We then proceeded to sort bam files and mark read duplicates using Picard v.2.20.4 (http://broadinstitute.github.io/picard/). Variant calling was conducted utilizing GATK v.4.1.1.0 (McKenna *et al.* 2010) following best practices (DePristo *et al.* 2011, Van der Auwera & O'Connor 2020). First, we employed Haplotypecaller with -ERC GVCF enabled on the duplicate marked bam files. The resultant gVCFs were combined for each individual and chromosome

using CombineGVCFs, and then jointly genotyped using GenotypeGVCFs. Biallelic SNPs and INDELs were separately selected from the subsequent vcf with genotyped variants using SelectVariants. We applied "hard filtering" standards to filter the resulting VCFs for both SNP and INDEL variants. For SNPs, this involved filtering out variants with Phred quality (QUAL) < 30.0, root mean square mapping quality (MQ) < 40.00, strand odds ratio (SOR) > 4.00, variant quality by depth (QD) < 2, fisher strand bias (FS) > 60.00, mapping quality rank sum test (MQRankSum) < -12.5, and read position rank sum test (ReadPosRankSym) < -8.00. For INDELs, filtering criteria included (QUAL) < 30.0, MQ < 40.00, SOR > 10.00, QD < 2.00, FS > 200.00, and ReadPosRankSum < -20.00. Sex chromosomes and unplaced scaffolds were excluded form the final analysis, and genotypes were filtered for biallelic loci with missing rate < 5%, Hardy–Weinberg equilibrium p-value > 1e-6, and minor allele count > 16 using PLINK v1.9.0 (Chang *et al.* 2015) yielding a total of 6,024,856 variants.

Individual pairwise relatedness was assessed using PLINK2. Several samples appeared to be closely related and were found to have an abnormally negative inbreeding metric (F). It was determined that the patterns of relatedness aligned with a pattern of inter-sample contamination most likely resulting from index hopping. To further assess this, CHARR was utilized. Samples with contamination rates over 2.8% were disregarded from downstream analysis. This threshold was chosen to balance previous best practices (Bick *et al.* 2024 advocates a 3% maximum allowance in research pipelines) and possible impact on statistical power. We found a quarter of our sequences included site contamination at levels between 1% and 2.65% and it bears mentioning that this may have contributed some unbiased noise to association signals.

A check of relatedness on the 90 retained individuals using vcftools --relatedness and --relatedness2 functions showed no close relations according to both A_{jk} statistics (Yang *et al.* 2010) and calculated relatedness Φ following the KING method (Manichaikul *et al.* 2010). Only one pair of individuals showed any potential relationship according to these methods (LM-13-122 and LM-18-052B; A_{jk}= 0.114 Φ= 0.099) although this was at a level below first-cousins.

Phenotypes:

In preparation for downstream analysis, several of the originally assessed phenotypes were examined to investigate genomic regions potentially associated with size and weight. These included: Non-eviscerated Weight (WeightNE), Total Fat (TF), Lean Dry Body Mass (LDBM), Wing Length (Wing), Head Length (Head), Sternum Length (SternL), and Sternum to Coracoid Length (SternLCorL). Sex and collection year (CollYear) information were included as covariates for some tests. Log transformations were applied to LDBM, TF, and WeightNE to normalize skewed distributions in some test iterations.

Genome Wide Associations:

Each analysis was conducted in GEMMA v0.98.3 (Zhou & Stephens, 2012) under a univariate linear mixed model, with sex and collection year included as covariates when appropriate (dummy-encoded at k–1 levels). The genomic relatedness matrix (GRM) was calculated from an LD-pruned subset of the filtered genotypes using GEMMA's mean genotype method. Association tests were performed using the likelihood ratio test (LRT). To account for multiple testing Benjamini–Hochberg correction was applied, with a false discovery rate (FDR) threshold prespecified at 10%.

To complement the genome-wide scan, we also carried out a more targeted analysis using previously identified fluctuating sites (Squires *et al.* 2026; in review). The variants, putatively under selection in the context of demographic cycling, were prioritized to examine whether they could clarify differential genotype-phenotype associations in response to changing population density. This followed an approach similar to Han *et al.* (2025). Specifically, we restricted the genotype dataset to the top 5% of fluctuating SNPs, as defined by the sum of absolute minor allele frequency changes across all collection years. This threshold was chosen partially to increase statistical power. Genotype filters were applied after subsetting to the top 5% of fluctuating loci, and testing was performed on a reduced set of 133,930 loci.

To explore potential genotype-by-environment (G×E) interactions, GEMMA was configured to test for G×E effects, with sex included as the sole covariate to avoid collinearity with year. A univariate model with population phase (peak or trough) as the target variable was tested with a Likelihood Ratio Test. The Benjamin-Hochberg procedure was used to control the False Discovery Rate. We performed additional GxE analyses for both phenotypes (TF and LDBM).

Results:

Phenotypic Data Trends:

Phenotype data was complete for all terms and analyzed with no missing values (Tab. S1). When looking across averages for each term, most appeared relatively stable across collection years. Initial investigations showed that there were no major outliers among terms (Fig. S1) and size metrics were unremarkable in terms of interannual fluctuations (Fig. 2). Upon separating phenotypic variables by sex it was clear that female birds showed consistently reduced values compared to males for all terms except for fat (Fig. 2). Notably fat levels were similar for male and female birds and more clearly fluctuated according to population cycle position (Fig. 2). A two-sample t-test indicated that there was no clear statistical difference in means for fat scores according to sex (F-test $p = 0.131$; t-test $p = 0.421$). LDBM was also observed to fluctuate

between years, however the interannual trend was more apparent in males (Fig. 2). Spearman correlations and pairwise plots revealed that collinearity between terms was moderate, with size related metrics and weight related metrics (Wing, Head, SternLCorL, LDBM, and TF) showing clear internal relationships(Fig S1). Notably, SternL and SternLCorL were highly similar ($R^2 = 0.91$) and LDBM was strongly correlated with WeightNE ($R^2 = 0.81$) while TF appeared uncorrelated to all terms and Head was mostly uncorrelated to LDBM ($R^2 < 0.5$; see all terms in Fig. S1).. The phenotype dataset was reduced to retain biologically meaningful, non-redundant traits for downstream GWAS. Final tests utilized the phenotypes of TF and LDBM which also showed the clearest annual fluctuations (Fig. 2).

Associations with SNPs:

After filtering, 6,024,856 SNPs were tested in the univariate GWAS and 133,930 SNPs in the G×E analyses. The latter utilized a targeted SNP approach, restricted to the top 5% of fluctuating outliers (Han et al. 2025). SNPs showed no apparent structuring based on sampling year or sex. the Benjamini–Hochberg procedure was used to account for multiple testing. No loci were significant at the prespecified 10% FDR threshold, with the minimum q-values being q = 0.2020 and q = 0.1160 for the GWAS and GxE analyses of total fat content respectively.

Our GWAS on log(TF) produced a best raw P-value of = $1.01*10^{-7}$ (adjusted P = 0.202161) for a SNP downstream of a Dead BOX Helicase gene (Fig. 3). 9,977 SNPs of 6,024,856 analyzed were found to be associated at P<0.001. For G×E on LDBM (Fig. S2), we saw a best raw P-value of $1.30*10^{-5}$ (adjusted P = 0.410876) in a SNP 21kb downstream from a predicted but undescribed gene on Chromosome 3. 269 SNPs of 133,930 analyzed were associated at P<0.001. For G×E on log(TF) we saw a best raw P-value of $8.69*10^{-7}$ (adjusted P-value = 0.116413; Fig. 4) in a SNP overlapping the HIVEP zinc finger 3 gene (*HIVEP3*; Fig. 5).

Of particular interest, the gene *HTR1F* accounted for approximately a third of the top one-hundred scoring SNPs, appearing 33 times (Fig. 5). By contrast, no SNPs from the G×E analysis on LDBM were present among the 100 lowest P-values (raw or adjusted) observed (Tab. S2 & S3).

Discussion:

Chitty (1957, 1967) was the first to suggest that there is a genetic component to the characteristics of population dynamics . He suggested that there are systematic changes in the genetics of the animals, environment quality and quantity of inter-actions between individuals). Cyclic population dynamics could result from density-induced changes in genetic traits affecting survival and/or reproduction of individual organisms (Chitty 1977, Henderson 1977). This study

presents the first exploratory genome-wide association analysis for key phenotypic traits in the Icelandic rock ptarmigan. By focusing sampling on juveniles collected from a single population in both peak and trough years, we were able to search for evidence of genetic markers tied to key life history traits. Our exploratory GWAS provides valuable insight into the phenotypic architecture of Icelandic rock ptarmigan and its relationship to population cycling. Though these materials have previously been thoroughly approached for investigation (Nielsen *et al.* 2012; Nielsen *et al.* 2014; Skírnisson & Nielsen 2019; Morrill *et al.* 2022; Dépré & Nielsen 2023) the present study makes new unpublished data available for analysis. Phenotypic traits related to body size and condition showed varying degrees of interannual fluctuation, with TF and LDBM exhibiting the strongest year-on-year shifts. These fluctuations were consistent with known demographic cycles. Notably, TF appeared to fluctuate independently of sex, while LDBM showed more pronounced variation in males. These patterns suggest that condition-related traits may be more sensitive to ecological context and sex specific hormone levels than morphometric factors that are more stable across generations.

Collinearity among traits was moderate to high for size and weight metrics, with Wing, Head, SternL, SternLCorL, and WeightNE forming a tightly correlated cluster. SternL and SternLCorL were nearly redundant ($R^2 = 0.91$), and LDBM was strongly correlated with WeightNE ($R^2 = 0.81$). In contrast, TF was largely uncorrelated with size-related traits and showed weak association with Head length ($R^2 < 0.5$), indicating that energetic reserves are varying independently of structural size and weight. We could find no statistical difference between the sexes for fat scores, in line with historical observations across cohorts.

It has previously been well established that Icelandic rock ptarmigan experience cyclical multi-annual shifts in population density (Gardarsson 1988, Nielsen & Pétursson 1995, Magnússon 2005, Guðmundsson 2015) and that several phenotypic traits of survival and fitness consequence are cycling in kind (Brynjarsdóttir *et al.* 2003; Nielsen, 2011; Dépré & Nielsen 2023; Gonzalez 2014; Holmstad *et al.* 2004). Most recently the biology of the Icelandic population has been put into further context through comparative work on other regional populations of ptarmigan (Holder *et al.* 1999, Kozma *et al.* 2015, Kozma *et al.* 2018, Kozma *et al.* 2019, Squires *et al.* 2025), and detailed genomic investigation of our focal population (Squires & Rödin-Morch *et al.* 2023, Squires *et al.* 2026 [in Review]). It has been shown that compared to other regional populations the Icelandic rock ptarmigan has higher than expected nucleotide diversity (Squires *et al.* 2025), and furthermore that at the domestic national level, ptarmigan in the northeast of Iceland appear to have healthy effective population sizes (N_e) and are unlikely to be strongly influenced by negative effects from drift (Squires *et al.* 2026 [in Review]). After historic population crashes at the beginning of the 21st century, there has most recently been a major rebound in ptarmigan numbers surpassing the highest census estimates in many decades

(Nielsen *pers. comm.*). This is most probably due to a hard crash in predatory Gyrfalcon populations from avian influenza (Iceland Monitor, 2025).

In the current work we proceeded with an initial GWAS using a restricted dataset of 90 juveniles sampled across peak and trough years. This exploratory analysis employed a univariate linear mixed model in GEMMA, incorporating sex and collection year as covariates, and tested over six million high-quality SNPs for association with condition-related traits. While no associations surpassed the 10% FDR threshold, the strongest signals were observed for TF under the standard GWAS framework. To complement this genome-wide scan, we implemented a reduced approach inspired by Han *et al.* (2025), focusing on the top 5% of SNPs that exhibited multi-annual allele frequency fluctuations. These targeted tests, as well as additional genotype-by-environment analyses, did not yield significant hits but highlighted a subset of candidate loci of potential biological interest. Together, these results underscore both the polygenic nature of condition traits in ptarmigan and the need for expanded sampling to achieve sufficient power for detecting robust associations.

Among the genes identified by our GWAS as putatively associated with cycling and body fat, we saw indications of the importance of genes involved in behavioral and developmental factors. The most strongly associated SNPs we identified within a gene were inside *HTR1F*, a coding region that has already been recognized for having potential importance in avian behavior. In the Mountain Chickadee (*Poecile gambeli*) *HTR1F* is associated with the development of the hippocampus, cognition, and memory (Branch *et al.* 2022). By virtue of conserved structures in chickens *HTR1F* has also been putatively associated with cognition, memory, learning, and rewards (Sun *et al.* 2021). Other top candidates we saw such as *DACH1* and *CNOT4*, were previously associated with brain and body development (Sackton *et al.* 2019; Trigila *et al.* 2025; Chen *et al.* 2011).

Among top hits from the GWAS on Log(TF), *GALNT9* was notable for previously being associated with adipose fat deposition in the livers of Chickens (Jin *et al.* 2017). Lipid metabolism and synthesis was also associated with the *ACSL3* and *EBF1* gene in Chickens (Tian *et al.* 2021; Chao *et al.* 2025) which we observed among top hits in our GxE on Log(TF). The latter is specifically involved in deposition of abdominal fat. To see some genes putatively associated with fat, among our top hits is reassuring.

This trend continued among the top GxE hits. In mammals *LAMA3* is associated with skin disorders, but in poultry it has reportedly been associated with intramuscular fat deposition (Tian et al., 2025) and cecal health (Li et al., 2025) perhaps providing a clear link between fat deposition and parasite loads in the Rock Ptarmigan. One of the most significant associations we identified among all tests was at a polymorphism overlapping the *HIVEP3* gene (raw p-value = $8.69*10^{-7}$; adjusted p-value = 0.116412813). In humans this has previously been associated

with autism spectrum disorders and brain development (Gonzalez-Mantilla et al. 2016; Stessman et al. 2017), while a recent study in Budgerigar supports the possibility of immune functions (De Meyst et al., 2025).

It is probable that through the fluctuation of alleles, rock ptarmigan in Iceland are responding genetically to changes in density and their environment through the adjustment of multiple genetic pathways that help them appropriately respond to ecological conditions. Given that fluctuations in population density are largely driven by predation, it is plausible that some of these genes have influence on movement and food seeking behaviors. In response to high predation risks, individuals must adjust foraging patterns to avoid predation, therefore in different years there could be added risks or benefits to being a risky eater or rapidly storing fat. Such adaptive adjustments are known from established biological theories of energy economics and predation risk (Ydenberg & Dill 1986, Lima & Bednekoff 1999). While we identify some sites of putative importance it is likely that complex phenotypes with multiple aspects of varying collinearity such as size and weight are highly polygenic and therefore strong signals from large effect loci are not expected to appear significant without extremely large sample sizes.

Our ability to investigate potentially important polymorphisms was affected by a need to balance inclusion of rare alleles with adequate representation of sites. Small sample size (n=90) undoubtedly contributed to our difficulty in identifying more significant relationships, and this highlights a need for more genomic data from more temporal cohorts in future investigations. GWAS is not an infallible method for identifying traits of importance, and this has been previously shown in a related ptarmigan species (Wenzel *et al.,* 2015). Our current results highlight possible associations and serve as a valuable stepping stone towards identifying candidates of clear interest. In addition to our findings in the present paper, recent research has suggested other sites of adaptive/cyclical significance to the Icelandic ptarmigan population (Squires *et al.* 2026, in submission). This preliminary work can pave the way for new sequencing efforts with the abundant tissues and phenotypic data available in Iceland. Future research should incorporate more physiological or health metrics and individuals to help clarify extant genotype-phenotype relationships in rock ptarmigan.

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Figures and Tables:

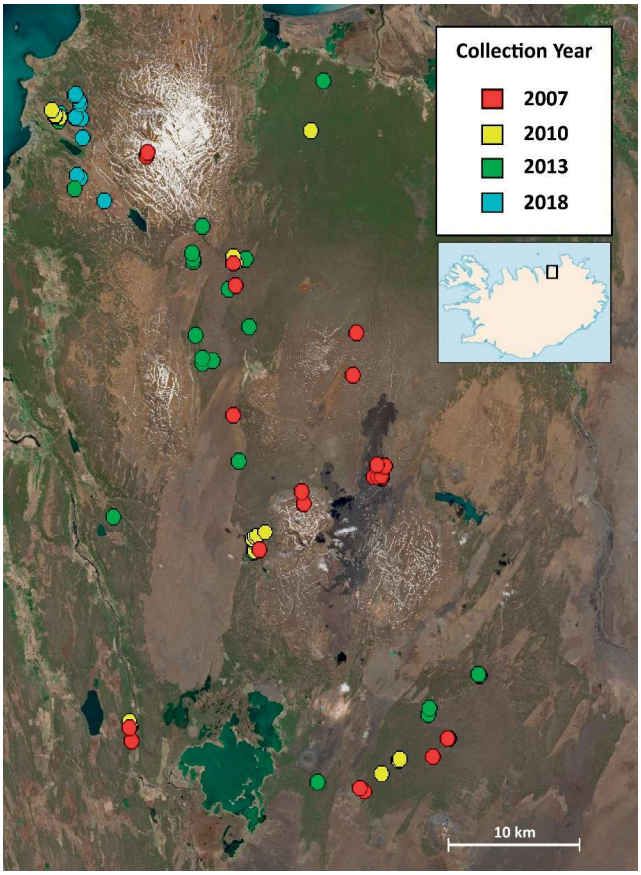

Figure 1. Sample collection locations overlayed on satellite imagery from the EU Copernicus Browser (European Space Agency 2025). The sampling area encompasses approximately 1500 km^2 in the northeast of Iceland.

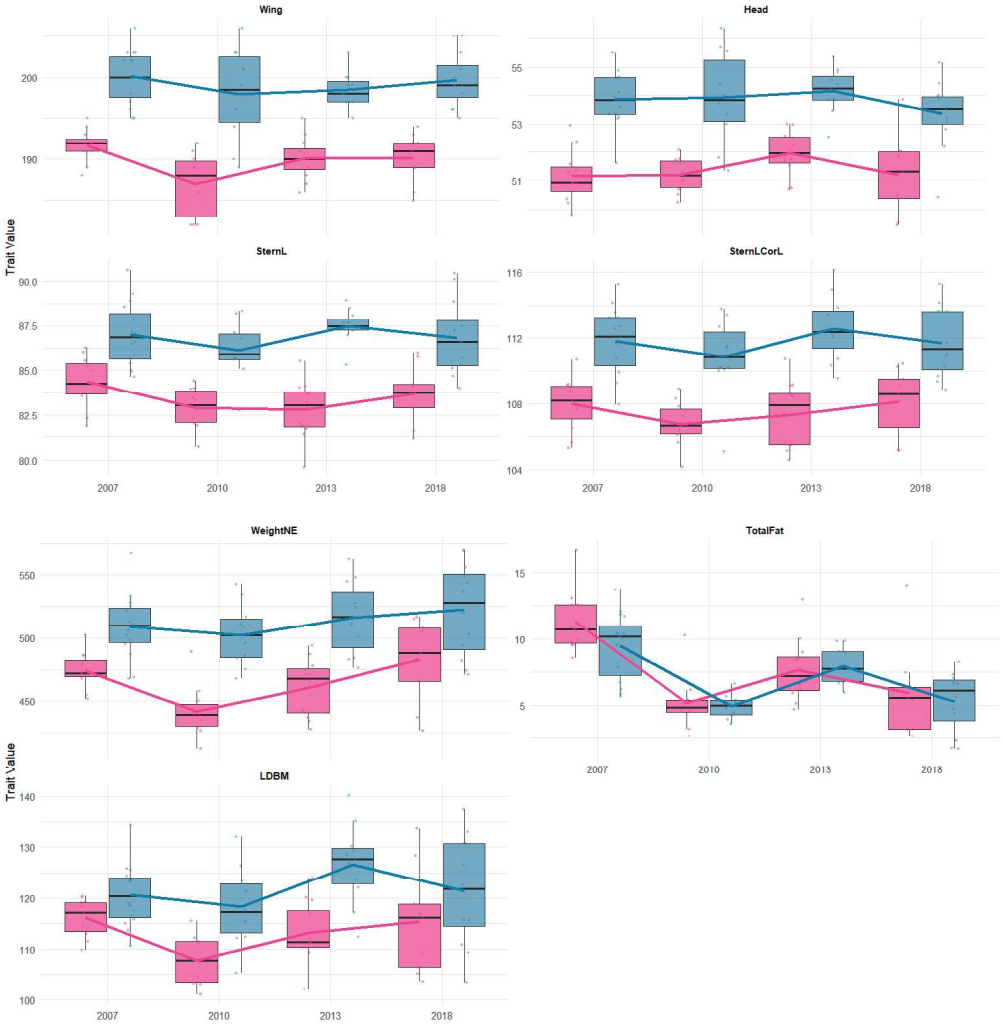

Figure 2. Trends of raw phenotype data across each collection year separated by sex. Total fat shows the clearest relationship with cycle position.

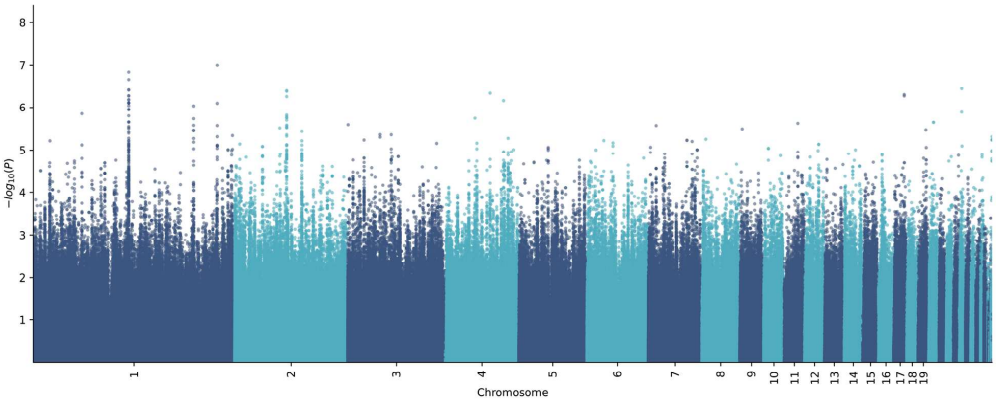

Figure 3. Manhattan plot displaying the association between 6,024,856 SNPs and log-transformed TF under a univariate linear mixed model in GEMMA. Sex and collection year (dummy-encoded at k–1 levels) were included as covariates, and the genomic relatedness matrix was calculated from an LD-pruned subset of genotypes. No SNPs surpassed the 10% false discovery rate (FDR) after Benjamini–Hochberg correction (best raw p-value = $1.01*10^{-7}$; best adjusted p-value = 0.202).

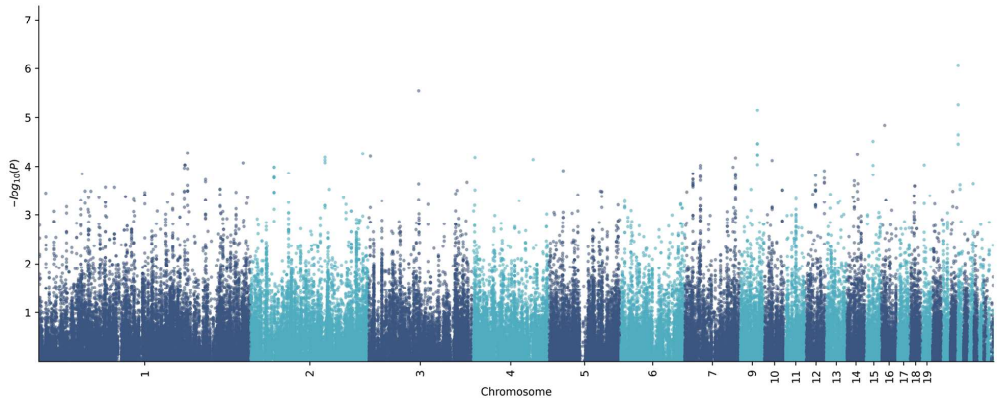

Figure 4. Manhattan plot displaying the association among 133,930 SNPS under a GxE on target phenotype comprised of log-transformed TF values with sex and collection year as covariates. No SNPs surpassed the 10% false discovery rate (FDR) after Benjamini–Hochberg correction (best raw p-value = $8.69*10^{-7}$; best adjusted p-value = 0.116).

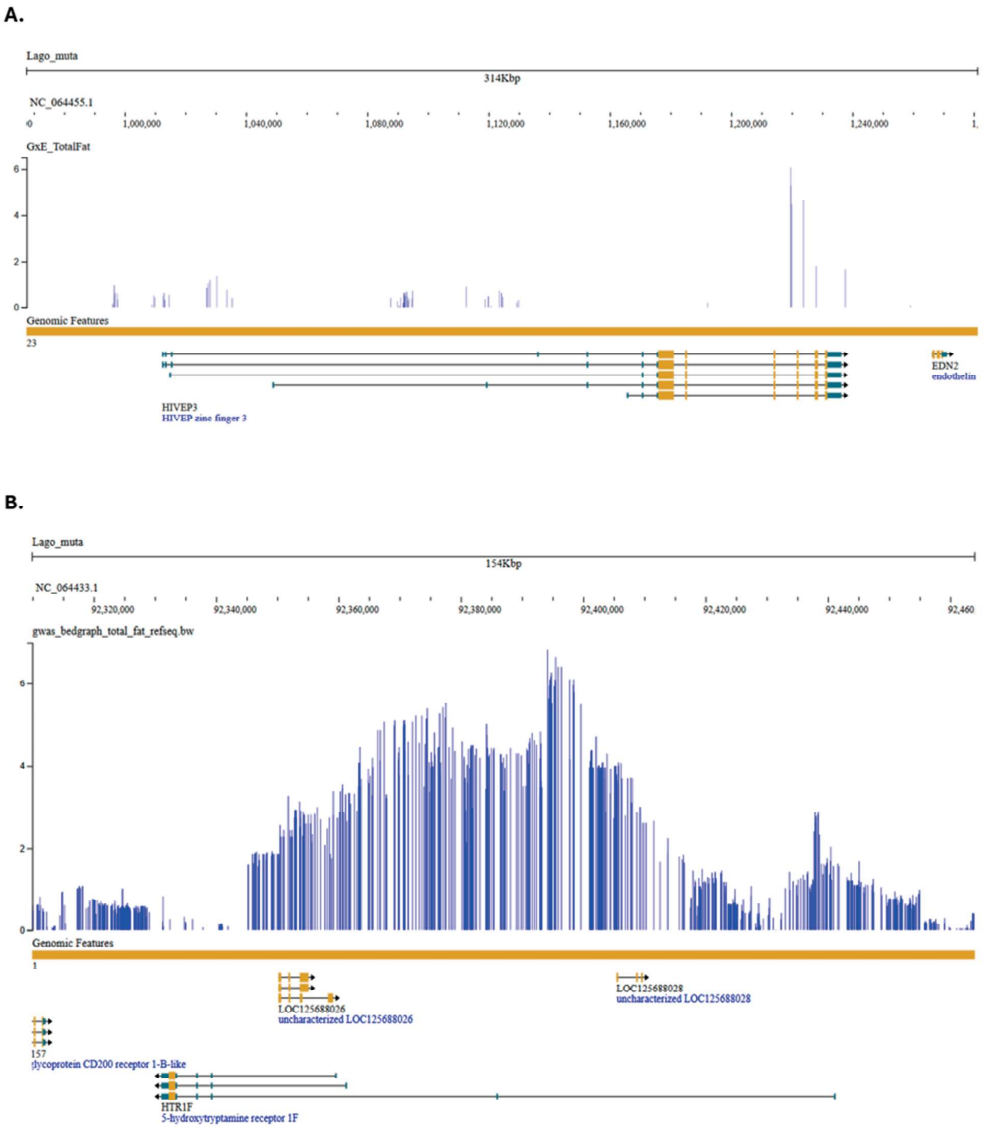

Figure 5. JBrowse2 output overlaying a GxE and GWAS track with gene annotations. (A.) For GxE on log(TF) the strongest peak observed was for an annotated gene region HIVEP3 (raw p-value = $8.69*10^{-7}$; adjusted p-value =0.116). (B.) For GWAS on log(TF) the best hit within a gene coincides with the annotated region for HTR1F (raw p-value = $1.47*10^{-7}$; adjusted p-value = 0.202).

Supplementary Figures and Tables

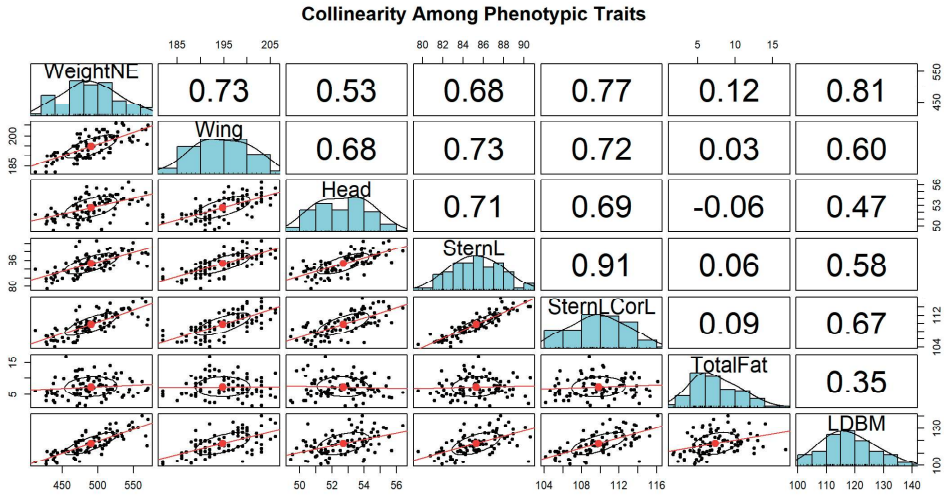

Figure S1. A paired panels assessment of exclusively phenotypic terms produced during the initial pre-analysis quality control investigation.

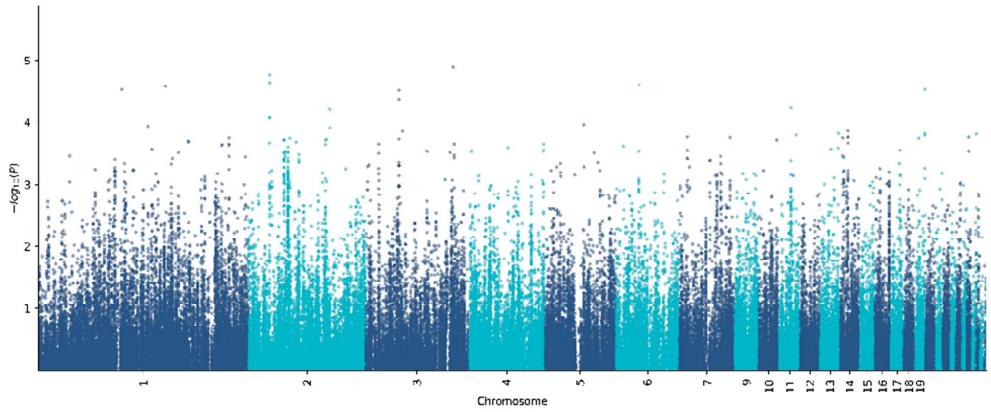

Figure S2 GxE on target phenotype comprised of log-transformed LDBM values with sex and collection year as covariates. No significant correlations were observed (λ_{GC}: 1.174; minimum adjusted P-value = 0.411)

BirdID	Latitude	Longitude	MASL	CollYear	Sex	WeightNE	Wing	Head	SternL	SternLCorL	TotalFat	LDBM
LM-07-005	65.60875	-16.6373	381	2007	F	452	191	51.3	83.76	105.33	9.874325	111.4576
LM-07-009	65.84391	-16.9929	360	2007	M	525	206	54.64	86.61	112.7	11.8712	134.3429
LM-07-010	65.94835	-16.9928	288	2007	F	455	192	51.58	85.49	108.79	16.72937	112.8711
LM-07-018	65.61993	-17.1718	240	2007	M	495	198	53.36	84.98	110.32	13.71545	115.0251
LM-07-019	65.9329	-16.9883	300	2007	M	468	195	54.11	85.84	109.91	10.36941	118.9963
LM-07-021	65.62148	-16.6092	370	2007	M	513	202	53.39	88.59	113.28	6.699514	118.4578
LM-07-028	65.58713	-16.7665	360	2007	F	502	192	52.38	84.95	110.66	10.7222	119.2656
LM-07-029	65.62157	-16.6103	370	2007	M	522	200	51.62	84.91	109.3	10.36321	124.3811
LM-07-031	65.62841	-17.1773	291	2007	M	498	203	55.52	90.63	114.15	10.20645	123.2368
LM-07-040	66.02276	-17.146	639	2007	F	471	194	49.8	81.87	105.66	12.55612	114.0827
LM-07-045	65.58429	-16.7583	360	2007	M	528	202	54.63	87.16	111.64	5.868172	123.5061
LM-07-046	65.75091	-16.9451	431	2007	F	473	191	50.22	86.02	109.2	13.045	120.2079
LM-07-051	66.02276	-17.146	639	2007	F	479	189	50.91	85.2	108.23	8.541696	120.4771
LM-07-052	66.02462	-17.1449	612	2007	M	487	200	54.65	86.87	110.3	5.694875	110.5826
LM-07-054	65.79143	-16.8695	671	2007	M	511	196	53.92	85.35	112.58	10.04555	116.5732
LM-07-056	65.78358	-16.8655	759	2007	F	486	193	52.94	86.27	109.04	10.7036	117.2463
LM-07-061	65.93298	-16.9883	300	2007	M	506	203	53.58	87.07	112.1	7.867114	113.6789
LM-07-066	65.80857	-16.723	474	2007	F	486	191	50.9	84.27	107.69	9.540134	114.4193
LM-07-071	65.87106	-16.7788	468	2007	M	469	195	53.17	89.32	113.28	10.36941	115.7655
LM-07-073	65.9003	-16.7719	437	2007	F	472	195	50.94	82.34	106.52	9.542201	118.3232
LM-07-082	65.80145	-16.7432	505	2007	M	533	197	53.85	88.91	113.59	12.04656	125.5927
LM-07-087	65.80968	-16.7362	462	2007	F	468	192	51.36	84.21	107.9	12.55612	109.7749
LM-07-091	65.8021	-16.7287	470	2007	M	510	200	53.24	84.64	107.99	11.69997	120.4098
LM-07-096	65.80096	-16.728	494	2007	M	502	202	53.32	86.49	110.73	9.536	125.8619
LM-07-099	65.8015	-16.7347	508	2007	F	471	188	50.36	83.56	109.11	10.87896	119.0636
LM-07-106	65.78322	-16.8671	767	2007	M	567	203	54.89	87.84	115.25	6.204429	123.708
LM-10-011	66.04923	-17.2995	305	2010	M	511	198	53.33	86.13	111.48	3.596607	126.3139
LM-10-012	66.05435	-17.3144	200	2010	F	427	182	51.32	81.92	106.74	4.581479	101.0109
LM-10-023	66.0503	-17.3075	357	2010	M	534	206	55.56	88.34	113.45	4.225955	123.3456
LM-10-027	66.0502	-17.3091	359	2010	F	458	191	50.24	83.47	106.07	3.211177	111.5325
LM-10-048	65.94893	-16.9872	295	2010	M	486	190	51.79	85.58	110.01	6.6124	117.5811
LM-10-053	65.60725	-16.6966	371	2010	F	450	189	51.76	82.03	106.39	4.48398	111.2875
LM-10-055	65.6066	-16.6975	373	2010	M	543	203	56.39	88.19	113.79	5.67028	132.1717
LM-10-065	65.75013	-16.9537	429	2010	F	412	182	51.72	83.98	108.34	6.19238	103.1309
LM-10-067	65.75839	-16.9566	420	2010	F	429	187	51.62	83.33	106.72	5.44313	104.5098
LM-10-091	65.76355	-16.9355	429	2010	M	475	201	51.34	85.07	110.16	5.17474	112.4209
LM-10-094	65.76101	-16.9497	426	2010	F	440	182	50.51	82.34	105.64	5.132648	103.2038
LM-10-100	65.63312	-17.1757	261	2010	M	496	199	53	86.8	111.4	5.368181	115.4074
LM-10-134	65.95315	-16.9916	275	2010	M	468	189	53.99	82.77	105.15	4.791687	105.266
LM-10-191	65.75955	-16.9544	428	2010	F	489	189	50.7	84	107.87	4.539387	115.4559
LM-10-199	65.75572	-16.9535	431	2010	F	439	192	50.97	82.77	104.14	2.646721	107.3747
LM-10-204	66.03998	-16.8542	121	2010	M	517	194	53.71	87.12	112.68	5.390497	121.382
LM-10-211	65.63312	-17.1757	261	2010	F	440	190	52.07	84.4	108.93	10.27961	112.0892
LM-10-215	65.62949	-17.1747	260	2010	F	435	186	51.05	80.73	107.26	5.273516	108.0808
LM-10-326	65.59693	-16.7288	378	2010	M	509	203	54.41	85.65	110.16	4.446616	117.0045
LM-10-327	65.59681	-16.7289	378	2010	M	484	196	55.7	85.6	110.21	3.95015	112.211
LM-13-001	65.9559	-17.0657	282	2013	F	471	186	51.66	84.06	107.43	5.149502	110.3319
LM-13-010	65.94959	-17.0628	306	2013	F	487	189	52.24	83.75	108.46	10.01063	116.919
LM-13-011	65.90465	-16.9631	330	2013	F	465	195	52.99	81.91	105.16	7.781053	111.2881
LM-13-015	65.66514	-16.5557	373	2013	M	524	198	52.53	87.66	112.35	7.945514	123.621
LM-13-021	65.99995	-17.2749	388	2013	F	475	190	52.96	82.88	109.15	8.40052	120.1043
LM-13-028	66.04649	-17.3029	430	2013	M	517	200	54.55	88.92	114.93	6.750434	129.2835
LM-13-030	65.64184	-16.6432	375	2013	M	527	200	53.46	86.95	112.25	9.860819	127.7031
LM-13-039	65.63776	-16.6454	376	2013	F	437	187	50.74	81.65	105.53	8.509661	111.0305
LM-13-057	65.93038	-16.9992	265	2013	F	471	190	52.52	83.69	108.46	6.647077	112.0433
LM-13-075	65.6646	-16.5541	378	2013	M	548	197	53.83	88.49	112.44	9.014686	135.1704
LM-13-083	65.95191	-17.063	313	2013	F	434	190	52.58	82.07	105.63	6.512214	109.2461
LM-13-092	65.97418	-17.0473	286	2013	F	478	193	52.07	84.13	109.11	12.98019	124.0259
LM-13-098	65.75462	-16.9539	438	2013	M	501	199	54.38	88.1	113.86	7.760808	122.0712
LM-13-099	65.59142	-16.8417	340	2013	M	545	198	53.85	87.28	113.44	9.843877	128.6198
LM-13-103	65.81249	-16.9813	412	2013	M	562	203	53.88	87.67	116.13	9.050377	140.2278
LM-13-109	65.88141	-17.0287	373	2013	F	494	191	51.87	85.5	110.69	9.005423	119.613
LM-13-113	65.74924	-16.9549	429	2013	M	476	197	54.25	85.27	110.3	6.892976	112.4019
LM-13-115	65.77382	-17.2049	343	2013	M	511	199	55.37	87.32	112.03	7.74228	130.1429
LM-13-122	65.89899	-17.0594	318	2013	F	443	192	51.53	81.38	105.48	5.925751	110.3706
LM-13-123	65.88326	-17.047	336	2013	F	442	188	51.76	83.21	108.48	4.665779	102.0237
LM-13-128	65.87982	-17.0468	332	2013	F	428	190	50.71	79.54	104.62	6.207032	111.5049
LM-13-142	65.95196	-16.9697	297	2013	M	484	195	54.92	87.49	110.69	5.963115	117.2007
LM-13-147	66.07384	-16.8313	75	2013	M	483	197	54.82	87.23	109.55	6.682856	126.9085
LM-18-002	66.00918	-17.2702	350	2018	F	517	192	50.5	84.07	110.25	14.06256	133.6963
LM-18-003	66.00918	-17.2702	350	2018	M	556	198	53.57	85.64	111.69	5.624841	130.6514
LM-18-007	66.00918	-17.2702	350	2018	M	494	195	52.18	84	110.22	7.286878	122.6569
LM-18-018	65.95155	-17.0627	300	2018	F	488	194	51.31	85.97	110.44	6.363815	116.958
LM-18-022	66.00713	-17.26411	367	2018	F	483	189	52	83.75	108.18	7.468193	128.2506
LM-18-026	66.00712	-17.2641	380	2018	M	503	196	53.95	85.09	109.37	8.235178	115.6252
LM-18-036	65.99182	-17.2216	298	2018	M	569	196	50.43	84.65	109.68	6.675882	115.6917
LM-18-037	65.99182	-17.2216	298	2018	M	544	199	53.2	86.56	113.63	6.609713	133.0522
LM-18-046	66.04794	-17.2628	296	2018	F	505	193	49.54	84.21	108.63	2.647514	109.1225
LM-18-052	66.04794	-17.2628	296	2018	F	466	186	51.85	81.56	105.19	5.559862	106.4366
LM-18-058	66.06498	-17.2728	265	2018	M	474	199	53.92	90.43	114.16	2.327768	103.4513
LM-18-063	66.0512	-17.3004	327	2018	F	427	185	50.37	81.17	105.21	3.214656	103.6301
LM-18-066	66.05079	-17.3036	289	2018	M	471	201	52.82	86.61	110.87	1.696835	109.3399
LM-18-067	66.0512	-17.3004	327	2018	M	482	203	53.49	85.29	108.88	1.779858	110.9175
LM-18-069	66.06022	-17.2664	288	2018	M	549	200	55.18	90.12	115.28	4.374442	130.7569
LM-18-081	66.04911	-17.272	282	2018	F	508	189	52.02	83.68	109.17	5.37078	116.0978
LM-18-084	66.04879	-17.2745	285	2018	M	570	205	53.06	87.48	111.01	7.08862	137.3326
LM-18-089	66.05798	-17.2632	290	2018	M	536	205	54.01	88.85	113.68	4.728381	121.1436
LM-18-093	66.06405	-17.2731	264	2018	M	520	199	54.43	87.23	111.7	6.840652	126.6244
LM-18-096	66.06405	-17.2731	264	2018	F	437	191	49.43	82.91	106.58	3.195511	105.1355
LM-18-097	66.06405	-17.2731	264	2018	F	515	192	53.87	85.72	109.51	5.66062	118.9117

Table S1. Complete list of phenotypic measurements for analysis and sample ID information for the 90 rock ptarmigan used in the study as collated by author OKN.

Chromosome	Position	Effect β	Error	P-value	Test	Gene Name	Function	Distance from Gene	Direction	SNPs
SUPER_1	178186506	-0.3158933	0.0572455	1.01E-07	GWAS_Fat	DDX10	DEAD-box helicase 10	40478	downstream	4
SUPER_1	92394100	0.2931563	0.0537584	1.47E-07	GWAS_Fat	HTR1F	5-hydroxytryptamine receptor 1F	*		33
SUPER_24	2307744	-0.2489894	0.0468161	3.71E-07	GWAS_Fat	PM20D1	peptidase M20 domain containing 1	*		2
SUPER_2	50517381	-0.286362	0.0541476	4.14E-07	GWAS_Fat	TPD52L1	TPD52 like 1	*		15
SUPER_4	43307207	-0.273136	0.0535301	4.72E-07	GWAS_Fat	MTUS1	microtubule associated scaffold protein 1	*		
SUPER_17	10305602	-0.2814428	0.0536543	5.10E-07	GWAS_Fat	GALNT9	polypeptide N-acetylgalactosaminyltransferase 9	*		2
SUPER_4	56442690	-0.3708045	0.0735245	6.97E-07	GWAS_Fat	LDB2	LIM domain binding 2	*		
SUPER_23	1219550	0.5242267	0.1008801	8.69E-07	GxE_Fat	HIVEP3	HIVEP zinc finger 3	*		2
SUPER_1	155109515	-0.3980245	0.0791917	9.24E-07	GWAS_Fat	DACH1	dachshund family transcription factor 1	*		7
SUPER_1	47043000	0.2327639	0.0471201	1.43E-06	GWAS_Fat	CNOT4	CCR4-NOT transcription complex subunit 4	*		
SUPER_4	28754647	-0.2326126	0.0486771	1.83E-06	GWAS_Fat	ADGRL3	adhesion G protein-coupled receptor L3	*		
SUPER_20	4761274	-0.3334063	0.0691924	2.30E-06	GWAS_Fat	NCBP3	nuclear cap binding subunit 3	*		2
SUPER_11	13311279	-0.2553575	0.0542113	2.44E-06	GWAS_Fat	CADPS	calcium dependent secretion activator	-39544	upstream	
SUPER_3	485810	-0.232876	0.048575	2.63E-06	GWAS_Fat	LOC125690802	heat shock factor protein 1-like	-1070	upstream	
SUPER_7	7679087	-0.2710246	0.0570133	2.76E-06	GWAS_Fat	DPP6	dipeptidyl peptidase like 6	*		
SUPER_3	45849339	0.5115004	0.1079753	2.88E-06	GxE_Fat	LAMA3	laminin subunit alpha 3	5690	downstream	
SUPER_2	43728324	-0.2425057	0.0520983	3.15E-06	GWAS_Fat	CEP57L1	centrosomal protein 57 like 1	*		
SUPER_9	2513255	-0.3462519	0.0747476	3.33E-06	GWAS_Fat	LOC125697811	uncharacterized LOC125697811	22216	downstream	
SUPER_19	7658068	-0.2726245	0.0577222	3.47E-06	GWAS_Fat	OLFM1	olfactomedin 1	-37577	upstream	
SUPER_2	65079137	0.2591592	0.0555467	3.73E-06	GWAS_Fat	PLG	plasminogen	*		2
SUPER_3	31212998	-0.3414486	0.073659	4.43E-06	GWAS_Fat	ELOC	elongin C	-1407	upstream	2
SUPER_3	42150101	-0.2370376	0.0511688	4.44E-06	GWAS_Fat	ASXL3	ASXL transcriptional regulator 3	*		
SUPER_1	192843959	-0.3060348	0.0659127	4.64E-06	GWAS_Fat	LOC125688084	uncharacterized LOC125688084	*		
SUPER_37	51087	0.3985364	0.086106	4.94E-06	GWAS_Fat	PAF1	PAF1 homolog, Paf1/RNA polymerase II complex component	*		4
SUPER_4	60917047	0.2242215	0.049523	5.39E-06	GWAS_Fat	ABLIM2	actin binding LIM protein family member 2	*		
SUPER_8	3847594	-0.3617905	0.0779049	5.65E-06	GWAS_Fat	ARHGAP15	Rho GTPase activating protein 15	*		
SUPER_3	15827636	0.2258032	0.0487131	5.88E-06	GWAS_Fat	CSMD3	CUB and Sushi multiple domains 3	324624	downstream	
SUPER_7	37517319	-0.2865368	0.0622149	5.97E-06	GWAS_Fat	LOC125696119	uncharacterized LOC125696119	-37392	upstream	2
SUPER_6	16531894	0.2538134	0.0553981	6.06E-06	GWAS_Fat	CPT1A	carnitine palmitoyltransferase 1A	*		
SUPER_1	16071202	-0.2442865	0.0529093	6.12E-06	GWAS_Fat	TBC1D22A	TBC1 domain family member 22A	-86469	upstream	
SUPER_7	42620790	0.2135609	0.0464604	6.33E-06	GWAS_Fat	TRAK1	trafficking kinesin protein 1	*		
SUPER_6	25520385	0.2304835	0.0492384	6.86E-06	GWAS_Fat	ZNF106	zinc finger protein 106	*		
SUPER_4	30599723	0.2120324	0.0469537	6.96E-06	GWAS_Fat	MOB1B	MOB kinase activator 1B	*		
SUPER_3	86086250	-0.2250052	0.0494417	7.02E-06	GWAS_Fat	TRNAY-GUA	transfer RNA tyrosine (anticodon GUA)	-6664	upstream	
SUPER_9	15897436	-0.4617377	0.09958	7.17E-06	GxE_Fat	ACSL3	acyl-CoA synthetase long chain family member 3	-6631	upstream	

Table S2. Summary of genes present among the 100 lowest raw P-values for both GWAS and GxE tests. Genes that appeared multiple times in the list are noted by number of times they occurred under the SNPs column and the position for the lowest P-value among those loci is shown. An asterisk indicates that candidate SNPs appeared within a gene.

Chromosome	Position	Effect β	Error	P-Value Adjusted	Test	Gene Name	Function	Distance from Gene	Direction	SNPs
SUPER_23	1219550	0.524227	0.10088	0.116412813	GxE_TotalFat	HIVEP3	HIVEP zinc finger 3	*		4
SUPER_3	45849339	0.5115	0.107975	0.192990117	GxE_TotalFat	LAMA3	laminin subunit alpha 3	5690	downstream	
SUPER_1	1.78E+08	-0.31589	0.057246	0.202160629	GWAS_TotalFat	DDX10	DEAD-box helicase 10	40478	downstream	3
SUPER_1	92394100	0.293156	0.053758	0.202160629	GWAS_TotalFat	HTR1F	5-hydroxytryptamine receptor 1F	*		28
SUPER_24	2307744	-0.24899	0.046816	0.202160629	GWAS_TotalFat	PM20D1	peptidase M20 domain containing 1	*		2
SUPER_2	50517381	-0.28636	0.054148	0.202160629	GWAS_TotalFat	TPD52L1	TPD52 like 1	*		13
SUPER_4	43307207	-0.27314	0.05353	0.202160629	GWAS_TotalFat	MTUS1	microtubule associated scaffold protein 1	*		
SUPER_17	10305602	-0.28144	0.053654	0.202160629	GWAS_TotalFat	GALNT9	polypeptide N-acetylgalactosaminyltransferase 9	*		2
SUPER_4	56442690	-0.3708	0.073524	0.202160629	GWAS_TotalFat	LDB2	LIM domain binding 2	*		
SUPER_1	1.55E+08	-0.39802	0.079192	0.214137703	GWAS_TotalFat	DACH1	dachshund family transcription factor 1	*		5
SUPER_9	15897436	-0.46174	0.09958	0.240092126	GxE_TotalFat	ACSL3	acyl-CoA synthetase long chain family member 3	6631	upstream	6
SUPER_1	47043000	0.232764	0.04712	0.269561283	GWAS_TotalFat	CNOT4	CCR4-NOT transcription complex subunit 4	*		
SUPER_4	28754647	-0.23261	0.048677	0.290139466	GWAS_TotalFat	ADGRL3	adhesion G protein-coupled receptor L3	*		
SUPER_20	4761267	-0.33341	0.069192	0.308517747	GWAS_TotalFat	NCBP3	nuclear cap binding subunit 3	*		2
SUPER_11	13311279	-0.25536	0.054211	0.320019148	GWAS_TotalFat	CADPS	calcium dependent secretion activator	39544	upstream	
SUPER_3	485810	-0.23288	0.048575	0.320019148	GWAS_TotalFat	LOC125690802	heat shock factor protein 1-like	1070	upstream	
SUPER_7	7679087	-0.27102	0.057013	0.320019148	GWAS_TotalFat	DPP6	dipeptidyl peptidase like 6	5690	downstream	
SUPER_2	43728324	-0.24251	0.052098	0.326769076	GWAS_TotalFat	CEP57L1	centrosomal protein 57 like 1	22216	downstream	
SUPER_9	2513255	-0.34625	0.074748	0.334160605	GWAS_TotalFat	LOC125697811	uncharacterized LOC125697811	*		
SUPER_19	7658068	-0.27262	0.057722	0.341944528	GWAS_TotalFat	OLFM1	olfactomedin 1	*		
SUPER_2	65079137	0.259159	0.055547	0.350695195	GWAS_TotalFat	PLG	plasminogen	*		
SUPER_16	3232036	0.446479	0.101836	0.379443668	GxE_TotalFat	SLC35C2	solute carrier family 35 member C2	7486	downstream	
SUPER_15	7279625	-0.41981	0.101729	0.379443668	GxE_TotalFat	XYLT1	xylosyltransferase 1	*		2
SUPER_1	1.37E+08	-0.43417	0.103918	0.379443668	GxE_TotalFat	TUBGCP3	tubulin gamma complex associated protein 3	*		
SUPER_2	1.03E+08	0.457868	0.110489	0.379443668	GxE_TotalFat	C1D	C1D nuclear receptor corepressor	118960	downstream	
SUPER_14	10454821	-0.56286	0.139381	0.379443668	GxE_TotalFat	EBF1	EBF transcription factor 1	*		
SUPER_3	1460295	-0.57268	0.142546	0.379443668	GxE_TotalFat	EEF1D	eukaryotic translation elongation factor 1 delta	*		
SUPER_2	68701714	-0.49389	0.12163	0.379443668	GxE_TotalFat	SMOC2	SPARC related modular calcium binding 2	*		3
SUPER_4	3117973	0.401641	0.101285	0.379443668	GxE_TotalFat	FNIP2	folliculin interacting protein 2	*		
SUPER_7	46861415	0.516667	0.129244	0.379443668	GxE_TotalFat	BBS9	Bardet-Biedl syndrome 9	*		
SUPER_4	56633755	-1.01897	0.248521	0.379443668	GxE_TotalFat	TAPT1	transmembrane anterior posterior transformation 1	9310	upstream	
SUPER_10	7414275	0.628849	0.154376	0.379443668	GxE_TotalFat	FANCI	FA complementation group I	*		
SUPER_1	1.88E+08	-0.42571	0.109375	0.379443668	GxE_TotalFat	DLG2	discs large MAGUK scaffold protein 2	*		
SUPER_1	1.34E+08	0.426955	0.108367	0.379443668	GxE_TotalFat	LOC125688141	pinopsin-like	24940	upstream	3
SUPER_19	2424008	0.492648	0.126083	0.379443668	GxE_TotalFat	CACNA1B	calcium voltage-gated channel subunit alpha1 B	*		
SUPER_7	14726577	0.449917	0.11261	0.379443668	GxE_TotalFat	SVIL	supervillin	*		
SUPER_2	21769145	-0.43477	0.110906	0.379443668	GxE_TotalFat	CSMD1	CUB and Sushi multiple domains 1	*		2
SUPER_7	44536318	0.442306	0.113399	0.379443668	GxE_TotalFat	ARPP21	cAMP regulated phosphoprotein 21	*		

Table S3. Summary of genes present among the 100 lowest adjusted P-values for both GWAS and GxE tests. Genes that appeared multiple times in the list are noted by number of times they occurred under the SNPs column and the position for the lowest P-value among those loci is shown. An asterisk indicates that candidate SNPs appeared within a gene.