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USING GENETIC APPROACHES TO STUDY LOCAL ADAPTATION AND REPRODUCTIVE SUCCESS IN SNOW BUNTINGS (*PLECTROPHENAX NIVALIS*)

By

Keta K. Patel

A Thesis
Submitted to the Faculty of Graduate Studies
through the Great Lakes Institute for Environmental Research
in Partial Fulfillment of the Requirements for
the Degree of Master of Science
at the University of Windsor

Windsor, Ontario, Canada

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USING GENETIC APPROACHES TO STUDY LOCAL ADAPTATION AND REPRODUCTIVE SUCCESS IN SNOW BUNTINGS (*PLECTROPHENAX NIVALIS*)

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ABSTRACT

Genetic analyses can facilitate effective and timely conservation and management actions. Arctic-migratory species in particular are in need of conservation genetic insights as they are experiencing substantial population pressures due to the impact of climate change (and other anthropogenic effects) on processes that affect their survival and reproduction. Therefore, identification of genetic mechanisms driving population divergence and variation in reproductive fitness in such species is critical. The goal of this thesis is to examine reproductive isolation among breeding populations of an Arctic-migratory passerine, the snow bunting (*Plectrophenax nivalis*) and determine factors that drive local adaptation and variation in reproductive success in this species. Using neutral and functional genetic markers, I show substantial population isolation among six globally distributed snow bunting breeding populations that is primarily driven by high levels of genetic drift and stabilizing selection, but with divergent selection acting at key functional loci. While there were no significant predictors of within-pair reproductive success, I identify key male quality traits such as body mass, testosterone levels, and breast plumage as important drivers of extra-pair reproductive success, which ultimately contribute to realized fitness in snow buntings. My work highlights the population-specific responses that reinforce the importance of genetic variability of individuals and their subsequent reproductive outcomes. The information contained in this thesis, combined with the methodological approaches, will help direct conservation efforts at the among- and within-population levels to maintain genetic diversity and adaptive potential as rapid environmental change continues to threaten Arctic-migratory species.

DEDICATION

To my mom, dad, and my younger brother

for unconditional love and support,

endless sacrifices, and incredible patience.

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CHAPTER 1

GENERAL INTRODUCTION

Climate change

Arctic ecosystems are experiencing climate change at about twice the rate of the global average (Wauchope et al., 2017; Canosa et al., 2020). Consequently, Arctic migratory species are highly vulnerable to the detrimental impacts of increasing global temperatures at their breeding grounds. For example, an increase in winter temperatures can result in earlier springs, causing disruption of the onset of migration from wintering grounds to the breeding grounds (Fossøy et al., 2014). With such changes in migratory patterns and timing of arrival to the Arctic, affected avian species can also face changes in local food availability (McKinnon et al., 2016). Generally, timing of reproduction is strongly correlated with the annual peak of resource availability (Mayor et al., 2017). To maximize fitness, individuals must synchronize their breeding phenology (i.e. arrival time, finding a mate, egg laying, etc.) in a way that local food availability at the breeding grounds matches the peak of demand from growing offspring (Thomas et al., 2001; Visser et al., 2006; Visser et al., 2012). An additional challenge to this synchronization is that there are vastly different impacts of climate change on wintering and breeding grounds (Both et al., 2010). Therefore, individuals may be unable to adjust the onset of migration from their wintering grounds in a way to match arrival timing to resource availability on the breeding grounds (Pearce-Higgins et al., 2005; Clausen & Clausen, 2013). This mismatch in phenology can have negative impacts on their reproductive success (Post & Forchhammer, 2007; Bowers et al., 2016; Walker et al., 2019),

ultimately leading to population declines and potentially extirpations, leading to biodiversity loss in the Arctic (Both et al., 2006; Wood & Kellermann, 2015).

Genetic diversity and selection

Protection of biodiversity should happen at a level of ecosystems, species and genes, with an aim to retain diversity at all three levels (McNeely, 1994). Total genetic diversity and standing genetic variation can indicate a species' ability to adapt to environmental change and a lack of genetic diversity in quantitative traits may increase species' risk of extirpation or extinction (Barrett & Schluter, 2008). Unfortunately, genetic diversity is often overlooked as primary attention is given to geographical areas, ecosystems, ecological communities and/or species (Laikre et al., 2010; Coates et al., 2018) during conservation and management decision making. There are many factors that shape genetic variation within species: genetic drift, selection, mutation, gene flow, hybridization, introgression, and recombination (Edwards et al., 2016). Most populations do not have a genetically uniform distribution and therefore warranting knowledge of genetic population isolation to accurately identify units valuable for conservation of genetic diversity (Cutter & Payseur, 2013). Therefore, it is useful to identify divergent genetic populations within a given species. In general, for populations to persist amidst environmental changes, the individuals must have adaptive phenotypes that match their local environment (Fox et al., 2019; Xue et al., 2019).

Local adaptation

Population persistence depends on the processes that govern survival and reproduction of individuals in their local environment, and therefore these processes are critical in understanding the immediate and future impacts of climate change (Grazer & Martin,

2012). Individuals within a population may differ widely on how they respond to variation in their local environment (Hoffman & Hercus, 2000; Barrett & Schuluter, 2007; Jump et al., 2009) Local adaptation occurs when populations evolve through natural selection to be more fit in their local habitat than any other potential habitat (Kaweki & Ebert, 2004; Wellband, 2012). Consequently, individuals evolve specific traits that are advantageous based on their local environment, irrespective of the fitness of these traits in other habitats (Kawecki & Ebert, 2004). The local environment can have diversity of complex impacts on survival and reproduction of individuals (Møller et al., 2010; Pettorelli, 2012). As such, local adaptation shapes many traits that directly or indirectly relate to survival and/or reproduction. For example, in the great tit (*Parus major*), there is intraspecific variation in the size of melanin-based black stripe (i.e. tie width) present on males' belly which is associated with personality and survival (Senar et al., 2014), as well as breeding success (Norris, 1990a,1990b). Senar et al. (2014) has shown that divergent selection on this phenotype is driven by local adaptation; survival in forest-environment increases with larger stripe (directional positive selection), whereas survival in urban environment increases with smaller stripe size (directional negative selection). Ideally, local adaptation is best estimated through reciprocal transplant experiments; measuring fitness of individuals in their own habitat versus that when transplanted in other habitats (Blanquart et al., 2013). Although useful in some scenarios (i.e. plants), this approach is not often feasible in many organisms due to logistical constraints in many natural systems (i.e. some organisms are hard or even impossible to transplant without harm, some organisms are long-lived that adequate fitness measures

are impractical to obtain), which makes a genetic approach to examining local adaptation much more practically effective in addressing relevant research questions.

Using genetic tools to understand local adaptation

Despite the power of using genetics to study local adaptation, there is a lack of understanding of the underlying genomic patterns associated with local adaptation, as many local adaptation studies primarily focus on phenotypes rather than the underlying genetic variation and architecture (Kawecki & Ebert, 2004). Neutral genetic markers (i.e. microsatellite or mitochondrial locus sequence) have long been used for characterizing genetic diversity and population differentiation (Zimmerman et al., 2019; Ouborg et al., 2010); however, they may not always fully reflect genome-wide variation (Hedrick, 2001; He et al., 2016) and all differences observed among populations at these loci are often assumed to be neutral, which may not always be the case (Gemayel et al., 2012). While increasing the number of loci analyzed would likely cover larger portions of the genome, most the of diversity observed may not be truly functional or biologically meaningful (Luikart et al., 2003; Beaumont & Balding, 2004). Alternatively, studying genetic variation at functional coding loci and pairing it with associated environmental variation is a very powerful method to characterize patterns of local adaptation (Tiffin & Ross-Ibarra, 2014; Hoban et al., 2016), and, ultimately, address complex evolutionary questions (Kaweki & Ebert, 2004; Savolainen et al., 2013).

Recent technical advances in molecular genetics allow the use of rapid and inexpensive assays to study functional diversity through High Throughput Sequencing (Reuter et al., 2015). Current genomic research methods associated with functional diversity are largely focused on genome-wide association studies aimed at detecting key

single nucleotide polymorphisms (SNPs; single base-pair differences in DNA sequence) (Korte & Farlow, 2013). Some reduced-representation sequencing approaches simplify the overall process by reducing the overall genomic data using restriction enzyme digestion (i.e., RADSeq, ddRAD) (Wright et al., 2019). One form of reduced-representation sequencing involves analysis of candidate genes following a reference genome/transcriptome assembly. For example, Wellband et al. (2018) studied adaptive potential of different fish species using SNP-containing loci derived from *de novo* transcriptome assembly. Functional SNPs associated with specific genes are biallelic and can be located in either coding regions of the genes, intergenic regions, or in introns (Jukema & Agema, 2001). SNPs within coding-regions (i.e. derived from transcriptome) are further divided into synonymous and non-synonymous variants. Synonymous variants code for the same amino acid in the protein sequence (despite sequence differences) while non-synonymous (also known as missense) variants result in a different amino acid in the protein sequence. Hence, non-synonymous variants are most likely to represent functional genetic variation. Overall, SNPs located within coding regions are therefore useful in studying relationships between environmental variation and potentially adaptive genotypes (Hoban et al., 2016). Inferred adaptive genotypes can be related to phenotypes through characterization of the function of the SNP locus region. Taken together, functional SNPs that provide increased precision in studying and identifying biologically meaningful variation, as well as standing genetic diversity within, and genetic differentiation among, populations provide some of the most promising genetic tools for studying genomic patterns of local adaptation (He et al., 2016).

Reproductive fitness and extra-pair paternity

Measuring fitness of a male exclusively based on the success of his focal reproductive effort can over- or underestimate his success if the potential for male promiscuity is not taken into consideration. These occurrences include cases where a male also mates outside of his socially monogamous pair, resulting in additional offspring outside his social nest (Westneat et al., 1990; Griffith et al., 2002). Promiscuous mating can lead to extra-pair paternity (EPP), with the resulting offspring known as extra-pair offspring (EPO) (Westneat et al., 1990). In contrast, within-pair paternity (WPP) includes the offspring that a male sires with his social mate. Although a common occurrence across a diversity of avian species (Brouwer & Griffith, 2019), the persistence and degree of EPP within a pair depends on the time and energy allocation of the social male between gaining WPP, seeking EPP, and his contribution to parental care (Westneat et al., 1990; Bonier at al., 2014; Kaiser et al., 2015). Additionally, female choice plays a major role in EPP rates (Griffith et al., 2002; Westneat & Stewart, 2003; Brouwer & Griffith, 2019) as females are generally expected to prefer highly ornamented males (Wells et al., 2016), allowing high quality males to obtain EPP, thus increasing their overall fitness. Climate change, along with associated breeding phenology changes, are expected to impact female choice and male breeding strategies, and thus indirectly impact EPP and EPO (Westneat & Stewart, 2003). The effects of environmental change on mating success are particularly important for Arctic species as they tend to have very short breeding seasons (and therefore only a single, short opportunity to breed in a given year) compared to species from the temperate regions (Forsman & Mönkkönen, 2003). To properly

characterize reproductive success under climate change stressors, EPP must be taken into account to obtain accurate assessments of total male fitness.

Strong sexual selection has driven individuals to evolve strategies to enhance their reproductive success, one of which is investment in EPP (Vedder et al., 2011; Chaine et al., 2015). Given the direct benefits that males receive by engaging in extra-pair copulations (Griffith et al., 2002; O'Brien & Dawson, 2011), EPP rates are hypothesized to be strong contributing factors underlying the evolution and maintenance of mating behaviours and variation in fitness (Griffith et al., 2002; Brouwer & Griffith, 2019). A portion of this variation exists due to female's mixed reproductive behaviour strategy; allocation between mating with the social male or seeking an extra-pair male (Griffith et al., 2002). Selection favouring female pursuit for EPP results in positive selection for particular male phenotypes. For example, several studies have shown aspects of male performance traits (e.g., song, morphology and age) are associated with males gaining high levels of EPP (Griffith et al., 2002; Akçay & Roughgarden, 2007). Although a general link between 'male quality' and the rates of EPP may be present across a diversity of avian species, there is nonetheless still an immense degree of intraspecific variation in male phenotype and both associated losses of paternity in social broods and gains in EPP (Griffith et al., 2002). Regardless of the nature of the relationship, EPP provides a basis for sexual selection on male phenotypes since EPP is related to various measures of male quality (Webster at al., 2007). As a result, male quality traits are not only important drivers of EPP variation, but of the overall reproductive fitness of an individual. Additionally, male reproductive patterns are expected to change under climate

change in Arctic-breeding birds (Hoset et al., 2014), affecting the rates of EPP and WPP, hence resulting in trade-offs in reproductive investment.

Male reproductive trade-offs have been studied with respect to social and ecological factors such as breeding synchrony and breeding density as these parameters vary with respect to changes in local habitat quality (e.g, food availability and vegetation density), leading to variation in opportunities for interactions between potential extra-pair mates (Bennett & Owens, 2002). For example, increased food availability is associated with an early increase in temperatures (Mayor et al., 2017). This can lead to a highly synchronized breeding effort in species with short breeding seasons (Hoset et al., 2004), where males face a trade-off in allocation of energy towards gaining WPP (i.e. high levels of mate guarding and parental feeding) at the expense of seeking EPP (Hoset et al., 2009). Alternatively, breeding synchrony facilitates the female to assess multiple males simultaneously as extra-pair mates (Westneat et al., 1990), possibly causing high EPP levels. Similarly, there are mixed reports on the relationship between breeding density and EPP incidences (Griffith et al., 2002; Brouwer & Griffith, 2019). Regardless of a general inter- and intraspecific relationships between EPP rates and breeding density/synchrony, exploring differential reproductive investment through assessment of EPP and WPP, will provide insight into potential for male reproductive flexibility, which may allow them to select optimal partners based on socio-ecological conditions.

Snow buntings

Snow buntings (*Plectrophenax nivalis*) are circumpolar Arctic-breeding passerines. They over-winter in temperate regions and arrive on their breeding grounds in low and high Arctic regions during the breeding season. Interestingly, they are known as one of the

earliest-arriving spring avian migrants to the Arctic where males arrive a few weeks earlier than females at the breeding grounds to establish and defend breeding territories when Arctic temperatures are generally around -30°C, food resources are covered by snow, and high occurrences of unpredictable severe storms (Montgomerie & Lyon, 2020). When females arrive at the breeding grounds, they build a nest in rocky cavities and produce a single clutch per season generally containing 5-7 eggs (Guindre-Parker et al., 2013a; Guindre-Parker et al., 2013b). Breeding habitats are variable among bunting populations with the proportion of rocky areas (for nesting) versus vegetated tundra (for feeding) varying widely (Montgomerie et al., 1983). Based on phenotypic categorization, there are four known subspecies of snow bunting identified on the basis on plumage, mandible, beak, and wing chord variation (Montgomerie & Lyon, 2020). Snow buntings are socially monogamous: males feed the females during incubation, and biparental chick provisioning is important for successful offspring rearing. However, some observational data suggest that the species may be genetically promiscuous (Espmark & Moksnes, unpublished data as cited in Hofstad et al., 2002; Hoset et al., 2014). While most populations worldwide are migratory, some Alaskan Island populations are endemic since they experience a moderate coastal climate year round. Christmas Bird Count from the Audubon Society data suggest North American populations have experienced significant population decline as population size has been decreased by more than 50% over the last 50 years (Montgomerie & Lyon, 2020). Although climate change may be the primary driver, there is a lack of studies identifying specific factors or mechanisms for this, as many potential causes (i.e. effects of pesticides/contaminants, habitat change, human/research impacts) are unexplored. It is crucial to take an intensive approach in

understanding and monitoring this species across the globe to halt serious declines and to reverse this trend.

While multiple present studies have explored the biology and ecology of this species, we know little about snow buntings at a genetic level. Their circumpolar distribution, long-distance migration capabilities, and our limited knowledge on migratory connectivity (Macdonald et al., 2012) for a majority of populations makes it challenging to map or identify reproductively isolated populations. Additionally, it is likely that the populations experience spatial and temporal variation in both wintering and breeding habitats. Given this species' dependence on a critical breeding period, I postulate that reproductive isolation, and possible local adaptation, may contribute substantially to variation in reproductive fitness. Consideration of genetic architecture, locally adaptive traits and reproductive fitness variation would aid in exploring the causes of population decline and conservation management efforts.

Overall objectives and rationale

The overall aim of this thesis is to assess reproductive isolation and determine factors that contribute to the local adaptation and variation in reproductive success of a highly migratory Arctic-breeding passerine, the snow bunting. Through two data chapters, I apply genetic analyses to study reproductive isolation and functional population divergence to characterize potential local adaptation, and variation in male breeding success predicted by various measures of male quality.

Arctic-migratory avian species are at risk of population decline or potential extirpation since climate change is drastically altering local environmental conditions, and indirectly, impacting reproductive biology and success (see details above). Therefore,

it is essential to characterize the link between local adaptation and reproductive success to highlight the importance of protecting and preserving genetically diverse units that perform well in response to environmental change.

The specific objectives associated with this thesis are to:

i. develop snow bunting species-specific hypervariable microsatellite markers
ii. assemble *de-novo* transcriptome using RNAseq data
iii. design multiple transcriptome-derived SNP primers to identify SNP variation in functionally relevant genes
iv. microsatellite genotype individuals from multiple breeding snow bunting populations
v. SNP genotype individuals from multiple breeding snow bunting populations
vi. assess reproductive isolation among multiple breeding populations using neutral microsatellite markers
vii. test for genetic divergence among multiple populations at neutral (microsatellite) and known-function gene polymorphisms (SNPs), and investigate the roles of genetic drift and natural selection in population differentiation patterns
viii. determine the link between male quality and variation in reproductive fitness in one breeding population

Overview of data chapters

Chapter 2 of this thesis applies population genetic analyses to assess population genetic divergence and levels of reproductive isolation to partition the roles of genetic drift and selection in snow buntings. More specifically, this data chapter uses a candidate gene

approach to assess genetic divergence at functional SNP loci and assesses overall signatures of selection, potentially due to local adaptation, among six breeding populations of *P. nivalis* populations. Since the factors important in individuals' ability to survive and reproduce are expected to be variable among Arctic-breeding populations, we expect populations to be reproductively isolated, and selection to be dominant over drift at our selected functional locus markers. This chapter also investigates genetic diversity and structure at species-specific neutral microsatellite markers and functional SNP loci. Furthermore, Chapter 2 compares patterns of divergence at known-function gene SNP loci, controlling for putatively neutral microsatellite genetic divergence, to infer neutral or selection-based processes driving snow bunting population divergence.

Chapter 3 of this thesis tests for the effects of factors that have been hypothesized to affect male reproductive fitness in a breeding population of snow buntings at Mitivik (East Bay) Island, Nunavut, Canada. More specifically, it examines important male quality traits as potential predictors of variation in reproductive success in this species. This data chapter uses snow bunting-specific microsatellite DNA markers to quantify the both within-pair and extra-pair reproductive success for individual males over two successive years. Ultimately, this approach is designed to assess the realized fitness, which is the total reproductive output (combination of within-pair and extra-pair), for each male in the population, and I test for correlations of that output and its components with diverse measures of male quality known to be important drivers of reproductive success in passerines (Griffith et al., 2002; Guindre-Parker et al., 2013a; Guindre-Parker et al., 2013b; Guindre-Parker & Love, 2014; Hoset et al., 2014).

Collectively, these two data chapters use genetic tools to answer two different, yet

cohesive questions regarding the mechanisms at the heart of how this species manages responses to fine- and large-scale variability in intrinsic and extrinsic environmental variability. Although the phenomenon of climate change is not novel considering Earth's geological history, the intensity and magnitude of changes associated with current climate change are very rapid (Huntley et al., 2006). The questions addressed in this thesis will aid in providing a baseline to assess the impact of climate change as increasing temperatures are expected to alter local habitat characteristics and consequently the reproductive behaviours and genetic diversity of this species. More importantly, the results obtained in this thesis will allow for improved design and implementation of snow bunting population management programs for conservation of healthy, stable, and genetically diverse populations that can withstand the forecasted changes associated with climate change.

References

Akçay, E., & Roughgarden, J. (2007). Extra-pair paternity in birds: review of the genetic benefits. *Evolutionary Ecology Research*, *9*(5), 855.

Barrett, R. D., & Schluter, D. (2008). Adaptation from standing genetic variation. *Trends in Ecology & Evolution*, *23*(1), 38-44.

Beaumont, M. A., & Balding, D. J. (2004). Identifying adaptive genetic divergence among populations from genome scans. *Molecular Ecology*, *13*(4), 969-980.

Bennett, P. M., & Owens, I. P. (2002). *Evolutionary ecology of birds: life histories, mating systems and extinction*. Oxford University Press.

Blanquart, F., Kaltz, O., Nuismer, S. L., & Gandon, S. (2013). A practical guide to measuring local adaptation. *Ecology Letters*, *16*(9), 1195-1205.

Bonier, F., Eikenaar, C., Martin, P. R., & Moore, I. T. (2014). Extrapair paternity rates vary with latitude and elevation in Emberizid sparrows. *The American Naturalist*, *183*(1), 54-61.

Both, C., Van Turnhout, C. A., Bijlsma, R. G., Siepel, H., Van Strien, A. J., & Foppen, R. P. (2010). Avian population consequences of climate change are most severe for long-distance migrants in seasonal habitats. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1685), 1259-1266.

Bowers, E. K., Grindstaff, J. L., Soukup, S. S., Drilling, N. E., Eckerle, K. P., Sakaluk, S. K., & Thompson, C. F. (2016). Spring temperatures influence selection on breeding date and the potential for phenological mismatch in a migratory bird. *Ecology*, *97*(10), 2880-2891.

Brouwer, L., & Griffith, S. C. (2019). Extra-pair paternity in birds. *Molecular Ecology*, *28*(22), 4864-4882.

Canosa, I. V., Ford, J. D., Mcdowell, G., Jones, J., & Pearce, T. (2020). Progress in climate change adaptation in the Arctic. *Environmental Research Letters*, *15*(9), 093009.

Chaine, A. S., Montgomerie, R., & Lyon, B. E. (2015). Sexual conflict arising from extrapair matings in birds. *Cold Spring Harbor Perspectives in Biology*, *7*(3), a017590.

Clausen, K. K., & Clausen, P. (2013). Earlier Arctic springs cause phenological mismatch in long-distance migrants. *Oecologia*, *173*(3), 1101-1112.

Cutter, A. D., & Payseur, B. A. (2013). Genomic signatures of selection at linked sites: unifying the disparity among species. *Nature Reviews Genetics*, *14*(4), 262-274.

Edwards, S. V., Potter, S., Schmitt, C. J., Bragg, J. G., & Moritz, C. (2016). Reticulation, divergence, and the phylogeography–phylogenetics continuum. *Proceedings of the National Academy of Sciences*, *113*(29), 8025-8032.

Forsman, J. T., & Mönkkönen, M. (2003). The role of climate in limiting European resident bird populations. *Journal of Biogeography*, *30*(1), 55-70.

Fossøy, F., Stokke, B. G., Kåsi, T. K., Dyrset, K., Espmark, Y., Hoset, K. S., ... & Moksnes, A. (2015). Reproductive success is strongly related to local and regional climate in the Arctic snow bunting (*Plectrophenax nivalis*). *Polar Biology*, *38*(3), 393-400.

Fox, R. J., Donelson, J. M., Schunter, C., Ravasi, T., & Gaitán-Espitia, J. D. (2019). Beyond buying time: the role of plasticity in phenotypic adaptation to rapid environmental change. *Philosophical Transactions of the Royal Society B*, 374(1768), 1-9.

Gemayel, R., Cho, J., Boeynaems, S., & Verstrepen, K. J. (2012). Beyond junk-variable tandem repeats as facilitators of rapid evolution of regulatory and coding sequences. *Genes*, *3*(3), 461-480.

Grazer, V. M., & Martin, O. Y. (2012). Investigating climate change and reproduction: Experimental tools from evolutionary biology. *Biology*, *1*(2), 411-438.

Griffith, S. C., Owens, I. P., & Thuman, K. A. (2002). Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology*, *11*(11), 2195-2212.

Guindre-Parker, S., & Love, O. P. (2014). Revisiting the condition-dependence of melanin-based plumage. *Journal of Avian Biology*, *45*(1), 29-33.

Guindre-Parker, S., Baldo, S., Gilchrist, H. G., Macdonald, C. A., Harris, C. M., & Love, O. P. (2013b). The oxidative costs of territory quality and offspring provisioning. *Journal of Evolutionary Biology*, *26*(12), 2558-2565.

Guindre-Parker, S., Gilchrist, H. G., Baldo, S., & Love, O. P. (2013a). Alula size signals male condition and predicts reproductive performance in an Arctic-breeding passerine. *Journal of Avian Biology*, *44*(3), 209-215.

Hedrick, P. W. (2001). Conservation genetics: where are we now?. *Trends in Ecology & Evolution*, *16*(11), 629-636.

Hoban, S., Kelley, J. L., Lotterhos, K. E., Antolin, M. F., Bradburd, G., Lowry, D. B., ... & Whitlock, M. C. (2016). Finding the genomic basis of local adaptation: pitfalls, practical solutions, and future directions. *The American Naturalist*, *188*(4), 379-397.

Hoffmann, A. A., & Hercus, M. J. (2000). Environmental stress as an evolutionary force. *Bioscience*, *50*(3), 217-226.

Hofstad, E., Espmark, Y., Moksnes, A., Haugan, T., & Ingebrigtsen, M. (2002). The relationship between song performance and male quality in snow buntings (*Plectrophenax nivalis*). *Canadian Journal of Zoology*, *80*(3), 524-531.

Hoset, K. S., Espmark, Y. N. G. V. E., Moksnes, A. R. N. E., Haugan, T., Ingebrigtsen, M., & Lier, M. (2004). Effect of ambient temperature on food provisioning and reproductive success in snow buntings *Plectrophenax nivalis* in the high arctic. *Ardea*, *92*(2), 239-246.

Hoset, K. S., Espmark, Y., Fossøy, F., Stokke, B. G., Jensen, H., Wedege, M. I., & Moksnes, A. (2014). Extra-pair paternity in relation to regional and local climate in an Arctic-breeding passerine. *Polar Biology*, *37*(1), 89-97.

Huntley, B., Collingham, Y. C., Green, R. E., Hilton, G. M., Rahbek, C., & Willis, S. G. (2006). Potential impacts of climatic change upon geographical distributions of birds. *Ibis*, *148*, 8-28.

Jukema, J. W., & Agema, W. R. P. (2001). The pharmacogenetics of atherosclerosis. In *Cardiovascular Genetics for Clinicians* (pp. 89-100). Springer, Dordrecht.

Jump, A. S., Marchant, R., & Peñuelas, J. (2009). Environmental change and the option value of genetic diversity. *Trends in Plant Science*, *14*(1), 51-58.

Kaiser, S. A., Sillett, T. S., Risk, B. B., & Webster, M. S. (2015). Experimental food supplementation reveals habitat-dependent male reproductive investment in a migratory bird. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1803), 20142523.

Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology letters*, *7*(12), 1225-1241.

Korte, A., & Farlow, A. (2013). The advantages and limitations of trait analysis with GWAS: a review. *Plant Methods*, *9*(1), 1-9.

Laikre, L., Allendorf, F. W., Aroner, L. C., Baker, C. S., Gregovich, D. P., Hansen, M. M., ... & Waples, R. S. (2010). Neglect of genetic diversity in implementation of the convention on biological diversity. *Conservation Biology*, *24*(1), 86-88.

Luikart, G., England, P. R., Tallmon, D., Jordan, S., & Taberlet, P. (2003). The power and promise of population genomics: from genotyping to genome typing. *Nature Reviews Genetics*, *4*(12), 981-994.

Mayor, S. J., Guralnick, R. P., Tingley, M. W., Otegui, J., Withey, J. C., Elmendorf, S. C., ... & Schneider, D. C. (2017). Increasing phenological asynchrony between spring green-up and arrival of migratory birds. *Scientific Reports*, *7*(1), 1-10.

McKinnon, E. A., Macdonald, C. M., Gilchrist, H. G., & Love, O. P. (2016). Spring and fall migration phenology of an Arctic-breeding passerine. *Journal of Ornithology*, *157*(3), 681-693.

McNeely, J. A. (1994). Protected areas for the 21st century: working to provide benefits to society. *Biodiversity & Conservation*, *3*(5), 390-405.

Møller, A., Fiedler, W., & Berthold, P. (2010). Effects of climate change on birds. *Ostrich*, *82*(3), 249-250.

Montgomerie, R. D., Cartar, R. V., McLaughlin, R. L., & Lyon, B. (1983). Birds of Sarcpa Lake, Melville Peninsula, Northwest Territories: breeding phenologies, densities and biogeography. *Arctic, 36*(1), 65-75.

Norris, K. J. (1990a). Female choice and the evolution of the conspicuous plumage coloration of monogamous male great tits. *Behavioral Ecology and Sociobiology, 26*(2), 129-138.

Norris, K. J. (1990b). Female choice and the quality of parental care in the great tit *Parus major*. *Behavioral Ecology and Sociobiology*, *27*(4), 275-281.

O'Brien, E. L., & Dawson, R. D. (2011). Plumage color and food availability affect male reproductive success in a socially monogamous bird. *Behavioral Ecology, 22*(1), 66-72.

Ouborg, N. J., Pertoldi, C., Loeschcke, V., Bijlsma, R. K., & Hedrick, P. W. (2010). Conservation genetics in transition to conservation genomics. *Trends in Genetics, 26*(4), 177-187.

Pauls, S. U., Nowak, C., Bálint, M., & Pfenninger, M. (2013). The impact of global climate change on genetic diversity within populations and species. *Molecular Ecology, 22*(4), 925-946.

Pearce-Higgins, J. W., Yalden, D. W., & Whittingham, M. J. (2005). Warmer springs advance the breeding phenology of golden plovers *Pluvialis apricaria* and their prey (Tipulidae). *Oecologia, 143*(3), 470-476.

Pettorelli, N. (2012). Climate change as a main driver of ecological research. *Journal of Applied Ecology, 49*(3), 542-545.

Post, E., & Forchhammer, M. C. (2008). Climate change reduces reproductive success of an Arctic herbivore through trophic mismatch. *Philosophical Transactions of the Royal Society B: Biological Sciences, 363*(1501), 2367-2373.

Post, E., Brodie, J., Hebblewhite, M., Anders, A. D., Maier, J. A., & Wilmers, C. C. (2009). Global population dynamics and hot spots of response to climate change. *BioScience, 59*(6), 489-497.

Reuter, J. A., Spacek, D. V., & Snyder, M. P. (2015). High-throughput sequencing technologies. *Molecular Cell, 58*(4), 586-597.

Savolainen, O., Lascoux, M., & Merilä, J. (2013). Ecological genomics of local adaptation. *Nature Reviews Genetics, 14*(11), 807-820.

Senar, J. C., Conroy, M. J., Quesada, J., & Mateos González, F. (2014). Selection based on the size of the black tie of the great tit may be reversed in urban habitats. *Ecology and Evolution, 4*(13), 2625-2632.

Thomas, D. W., Blondel, J., Perret, P., Lambrechts, M. M., & Speakman, J. R. (2001). Energetic and fitness costs of mismatching resource supply and demand in seasonally breeding birds. *Science, 291*(5513), 2598-2600.

Tiffin, P., & Ross-Ibarra, J. (2014). Advances and limits of using population genetics to understand local adaptation. *Trends in Ecology & Evolution*, *29*(12), 673-680.

Vedder, O., Komdeur, J., van der Velde, M., Schut, E., & Magrath, M. J. (2011). Polygyny and extra-pair paternity enhance the opportunity for sexual selection in blue tits. *Behavioral Ecology and Sociobiology*, *65*(4), 741-752.

Visser, M. E., Holleman, L. J., & Gienapp, P. (2006). Shifts in caterpillar biomass phenology due to climate change and its impact on the breeding biology of an insectivorous bird. *Oecologia*, *147*(1), 164-172.

Visser, M. E., te Marvelde, L., & Lof, M. E. (2012). Adaptive phenological mismatches of birds and their food in a warming world. *Journal of Ornithology*, *153*(1), 75-84.

Walker, W. H., Meléndez-Fernández, O. H., Nelson, R. J., & Reiter, R. J. (2019). Global climate change and invariable photoperiods: A mismatch that jeopardizes animal fitness. *Ecology and Evolution*, *9*(17), 10044-10054.

Walther, G. R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J., ... & Bairlein, F. (2002). Ecological responses to recent climate change. *Nature*, *416*(6879), 389-395.

Wauchope, H. S., Shaw, J. D., Varpe, Ø., Lappo, E. G., Boertmann, D., Lanctot, R. B., & Fuller, R. A. (2017). Rapid climate-driven loss of breeding habitat for Arctic migratory birds. *Global Change Biology*, *23*(3), 1085-1094.

Webster, M. S., Tarvin, K. A., Tuttle, E. M., & Pruett-Jones, S. (2007). Promiscuity drives sexual selection in a socially monogamous bird. *Evolution: International Journal of Organic Evolution*, *61*(9), 2205-2211.

Wellband, K. (2012). Gene transcription mediated local adaptation of Babine Lake tributary rainbow trout. *Electronic Theses and Dissertations*, Paper 370.

Wells, S. J., Safran, R. J., & Dale, J. (2016). Piecing together female extra-pair mate choice: females really do prefer more ornamented males. *Molecular Ecology*, *25*(15), 3521-3524.

Westneat, D. F. (1990). The ecology and evolution of extra-pair copulations in birds. *Current Ornithology*, *7*, 331-369.

Westneat, D. F., & Stewart, I. R. (2003). Extra-pair paternity in birds: causes, correlates, and conflict. *Annual Review of Ecology, Evolution, and Systematics*, *34*(1), 365-396.

Wood, E. M., & Kellermann, J. L. (Eds.). (2015). *Phenological synchrony and bird migration: changing climate and seasonal resources in North America* (Vol. 47). CRC Press.

Wright, B., Farquharson, K. A., McLennan, E. A., Belov, K., Hogg, C. J., & Grueber, C. E. (2019). From reference genomes to population genomics: comparing three reference-

aligned reduced-representation sequencing pipelines in two wildlife species. *BMC Genomics, 20*(1), 1-10.

Xue, B., Sartori, P., & Leibler, S. (2019). Environment-to-phenotype mapping and adaptation strategies in varying environments. *Proceedings of the National Academy of Sciences, 116*(28), 13847-13855.

Zimmerman, S. J., Aldridge, C. L., Oh, K. P., Cornman, R. S., & Oyler-McCance, S. J. (2019). Signatures of adaptive divergence among populations of an avian species of conservation concern. *Evolutionary Applications, 12*(8), 1661-1677.

CHAPTER 2

GLOBAL POPULATION STRUCTURE IN AN ARCTIC-MIGRATORY BIRD: DIVERSIFYING AND STABILIZING SELECTION CONSISTENT WITH LOCAL ADAPTATION

Introduction

Local adaptation occurs when individuals from a given population exhibit higher fitness in their local environment than in other environments (Kaweki & Ebert, 2004). Local adaptation is a global phenomenon that has been demonstrated in diverse taxa including plants, bacteria, birds, mammals and fish (e.g., Lambrechts et al., 1996; Fraser et al., 2001; Leimu & Fischer, 2008; Hereford, 2009; Gorter et al., 2016, among others). Since habitats are spatially and temporally variable, local environmental conditions determine which traits may be favoured by selection (Hoban et al., 2016). As such, site-specific differences in habitat characteristics can create heterogenous selective pressure leading to divergent selection at the phenotypic and, indirectly, genotypic levels, resulting in local adaptation (Kaweki & Ebert, 2004). Two important evolutionary forces that reduce local adaptation are gene flow and genetic drift. While limited gene flow results from reproductive isolation, high gene flow reduces the potential for local adaptation by diluting the favoured genotypes (via associated phenotypes) by introducing new alleles in the population (Lenormand, 2002; Blanquart et al., 2012). Genetic drift reduces local adaptation through random fluctuations in allele frequencies, and hence genotype frequencies, which may not be optimal for local conditions (Yeaman and Otto, 2011; Blanquart et al., 2012). Rapid environmental change generated by global climate change and other anthropogenic effects directly impact local environments and the locally adapted individuals inhabiting those changing environments (Atkins & Travis, 2010;

Valladares et al., 2014). Consequently, anthropogenic change may result in a de-coupling of standing locally adapted allele frequencies and the rapidly changing environment. Thus, assessing local adaptation in natural populations is critical for predicting the effects of changing environments and stressors on locally adapted populations (Fraser et al., 2011; Aitken & Whitlock, 2013).

Advances in molecular genetic technology allow ecologists and evolutionary biologists to study genetic variation and the conservation and management of individuals, populations, and species across diverse taxonomic groups (Kirk & Freeland, 2011). Examples include: the Sand Cress (*Arabidopsis lyrata*), Lake Whitefish (*Coregonus clupeaformis*), Heliconius Butterfly (*Heliconius melpomene*), Rough Periwinkle (*Littorina saxatilis*), among many others (Turner et al., 2010; Renaut et al., 2010; Ferguson et al., 2010; Galindo et al., 2010, respectively). Neutral molecular genetic markers (e.g., microsatellite DNA markers or mitochondrial sequence data) are widely used to quantify genetic diversity, gene flow and genetic differentiation among populations (Ouborg et al., 2010; Zimmerman et al., 2019). Those markers are useful in conserving biodiversity by facilitating the identification of formal conservation units such as evolutionary significant units, management units, action units and family nets (Wan et al., 2004). While those outcomes can be valuable, inferences derived from neutral genetic marker data do not inform wildlife conservation managers about potentially locally adapted functional genetic variation. Functional genetic variation is particularly important with the growing concern over the rapid rate of global environmental change due to anthropogenic pressures such as climate change, among others. If a species is unable to disperse or express phenotypic plasticity in the face of environmental change, their

survival will solely depend on rapid adaptation which is only possible if there is sufficient, and relevant, functional genetic variation present (Jump & Penuelas, 2005; Jump et al., 2009). The characterization of variation at functional loci (i.e. the genes that code for specific proteins) among populations provides insight into adaptive divergence among the populations (Luikart et al., 2003; Beaumont & Balding, 2004). Divergence in functional gene polymorphism frequencies is expected to evolve rapidly in response to natural selection, contrary to evolution by genetic drift alone (Kawecki & Ebert; 2004). Such characterization is often achieved using High Throughout Sequencing (HTS) which permits exceptional power to assess variation in DNA, mRNA and cDNA (Reuter et al., 2015). The most common approach to quantifying functional genetic diversity in large-scale ecological studies involves genotyping single nucleotide polymorphisms (SNPs; single base-pair changes in DNA sequence) through reduced-representation sequencing strategies (Toews et al., 2016). Reduced-representation sequencing methods such as genotyping-by-sequencing (GBS) and restriction-associated DNA sequencing (RADseq) are practical approaches since they involve sequencing only a subset of the genome and therefore they reduce the cost of sequencing per individual (Toews et al., 2016). Since GBS and RADseq approaches result in SNPs located randomly throughout the genome (i.e. within coding and non-coding regions), they tend to be dominated by non-coding variants which are putatively neutral markers (although linkage disequilibrium makes it difficult to categorize them as strictly neutral). To target functional SNPs, whole transcriptome data generated by RNA-Seq are best as they only include transcribed sequences, and specific function can be determined following transcriptome assembly. For example, Wellband et al. (2018) used RNA-Seq data to characterize functional SNPs

and studied invasion success of two gobiid species in the Great Lakes at functional SNP markers relative to expected neutral divergence generated by microsatellite marker data. They were able to identify signatures of divergent selection at specific SNP loci, suggesting rapid adaptative evolution in one of the two invasive species.

Bird species are widely known for their long-range migratory patterns, in some cases covering substantial portions of the globe (Sekercioglu, 2007; Rolland et al., 2014). Such migratory life histories make them interesting candidate species for local adaptation analyses because, although they are exposed to a wide range of environments, they should experience the strongest local selection pressures at their breeding grounds. As a result, genomic signatures of local adaptation should be most apparent at the breeding grounds. Although there is an abundance of published studies of local adaptation in birds, there is limited published work on genetic patterns of divergence that underlie the process of local adaptation (Kawecki & Ebert, 2004). The majority of research on local adaptation in non-migratory birds involves the adaptive divergence of song and morphology among isolated populations (e.g., Slabbekoorn & Smith, 2002; Branch & Pravosudov, 2015; Job et al., 2015; Graham et al., 2016;). Curiously, even though migratory birds are highly impacted by environmental changes (Both et al., 2006; Jonzén et al., 2006; Visser et al., 2015), very little is understood about their differential adaptive capacity, especially regarding the extent to which genomic variation is shaped by local environmental factors (Bay et al., 2018). In migratory birds, migration and breeding phenology are critical to an individual's reproductive fitness, yet there are only a few studies reporting signatures of selection at known-function gene loci in their breeding populations. In one such study, Kuhn et al. (2013) studied genetic differentiation in extant

and historical populations of the pied flycatcher (*Ficedula hypoleuca*), a long-distance migratory passerine. Kuhn et al. (2013) used neutral microsatellite and mitochondrial markers and a functional *Clock* gene marker to test for potential effects of global climate change on the genetic structure of populations. They provided evidence of stabilizing selection at the functional marker and suggested a pattern of local adaptation having a greater effect on population structure and genetic variation than recent climate change. In a related study on the same species, Lehtonen et al. (2012) showed two (*follistatin* and *SWS1 opsin*) of fourteen candidate genes involved in plumage colouration exhibited adaptive divergence among 17 distinct sites across the species' breeding range. This is one of the few published studies of migratory passerines that employed a targeted SNP screening approach that measured genetic diversity and differentiation. To the best of our knowledge, there has only been one published study of selection at genetic marker loci in an Arctic-breeding passerine. Contrary to the expectation of local adaptation at the breeding grounds, Tigano et al. (2017) concluded that adaptation to migratory routes or some other non-breeding ground-based environmental factor drove the pattern of differentiation at genome-wide SNP markers in thick-billed murres (*Uria lomvia*). Patterns of population differentiation in migratory bird species in general, and more specifically, in Arctic migratory avian species, have been vastly understudied, despite the potential for population connectivity to have serious implications for their conservation in rapidly changing environments (Macdonald et al., 2012). As migration and breeding phenology are impacted heavily by anthropogenic stressors, (Cotton, 2003; Gordo, 2007; Both et al., 2010, Gullett et al., 2013), it is crucial to study the local adaptation of

breeding populations to assess their potential for adaptation to anthropogenic/climate change on these populations.

Snow buntings (*Plectrophenax nivalis)* are small, Arctic-breeding passerines with a circumpolar distribution (Montgomerie & Lyon, 2020). Despite this species' global distribution, there are few known population-level differences in their life histories, with the exception of migratory versus non-migratory populations (e.g., island populations such as Aleutian and Pribilof Islands are non-migratory; Table 2.1). There are currently four subspecies of snow buntings categorized on the basis on phenotypic differences such as plumage, bill size and wing chord length (Montgomerie & Lyon, 2020). Snow bunting populations annually migrate between high Arctic breeding grounds and temperate wintering grounds (Macdonald et al., 2012; Snell et al., 2018;). During the breeding season, male snow buntings arrive at the breeding grounds 3-4 weeks earlier than females to gain access to high quality nesting sites among the rocky cavities in the tundra (McKinnon et al., 2016). Although most populations are migratory, some island populations as well as a high-altitude Scottish population of this species are non-migratory. For example, some Alaskan island residents are non-migratory, as most individuals over-winter in their breeding range likely due to moderate climate throughout the year (Montgomerie & Lyon, 2020). While globally abundant, evidence from long-term winter census data suggests North American snow bunting populations have undergone substantial decline, with a reduction of 64% over the past five decades (Butcher & Niven, 2007). However, conservation efforts are hampered by many factors, including a lack of information on the basic population structure and selection pressures on the birds.

To address population structure and functional divergence consistent with local adaptation, we assessed global population structure and patterns of genetic divergence among six geographically-isolated breeding snow bunting populations. We first used microsatellite (presumed neutral) and transcriptome-derived SNP locus markers (functional) to determine genetic divergence and hence assess whether the sampled populations are reproductively isolated. We then investigated population genetic divergence at functional loci, controlling for the effects of genetic drift using the neutral microsatellite markers. More specifically, we employed genetic differentiation outlier detection to identify whether i) there was an overall signature of stabilizing versus divergent selection, and ii) there were specific genes that are responsible for functional divergence patterns using pairwise comparisons between specific populations. As a largely migratory species, snow buntings are expected to have widely dispersed breeding populations across the globe (Montgomerie & Lyon, 2020) and current (although limited) data suggests those populations which have been studied have generally consistent migratory patterns (Lyngs, 2003; Macdonald et al., 2012; Snell et al., 2018; Montgomerie & Lyon, 2020). Hence we predict reproductive isolation among the six breeding populations based on the expectation of consistent and separate migration routes; however, we recognized that including populations of essentially unknown migration behaviour may drive unexpected gene flow resulting in unexpected connectivity among some populations. We also predicted strong local selection pressures at the breeding grounds to result in patterns of local adaptation that would contribute to genetic differentiation at functional gene loci. This is based on the expectation that functional gene allele frequency differences will contribute to reproductive fitness of individuals.

Specifically, we hypothesized that snow buntings are adapted to the local conditions on their breeding grounds. This is driven by selection pressures being strongest during the breeding period due to the high energetic demands of breeding, a short seasonal breeding season, and a correlation between local and regional climate and reproductive success (Falconer et al., 2008, Fossøy et al., 2014, Hoset et al., 2014). Furthermore, we predicted a majority of our selected functional genes to be under genetic drift, with key functional genes under divergent selection but relatively few genes under stabilizing selection. In this study we describe powerful genetic approaches that can be used in future studies for the conservation and management of globally migratory species with the goal of facilitating the preservation of biodiversity.

Methods

This project included the development and application of two types of molecular markers: neutral microsatellite markers and functional gene locus SNP markers. It thus involved two types of samples: RNA samples for *de-novo* transcriptome assembly for SNP marker development, and DNA samples collected across the global breeding range of snow buntings for genotype data for the population genetic analyses. The population genetic study involved genotyping all samples at both microsatellite and SNP locus markers to determine population genetic divergence and patterns of functional divergence.

Development of microsatellite markers

To develop snow bunting-specific microsatellite markers, multiple heterospecific primers were screened, and primers chosen for strong amplification and high polymorphism on test samples (specifically, Mitivik Island DNA were used as a high-quality benchmark

DNA for primer optimization). Some primer sequences were modified using the species-specific sequence information from an unrelated Next Generation sequencing project.

DNA sample collection and extraction

For the population-level analyses, a large-scale collaborative effort collected snow bunting tissue from populations across a wide geographic range, resulting in a total of 221 samples for DNA extraction from individuals from six populations worldwide (Figure 2.1, Table 2.2). With the exception of the samples from Barrow, AK, USA, which were DNA extracted using QIAamp DNA Mini Kit (Qiagen Inc., Toronto, ON, Canada) as per manufacturer's instructions, all samples were extracted using a DNA extraction approach using solid phase reversible immobilization (SPRI) beads (Vo & Jedlicka, 2014). The SPRI beads extraction protocol was originally optimized for bird cloacal and oral swab samples. Briefly, this protocol involves the processing of samples in a solution containing lysis buffer, protein precipitation solution and zirconia-silica beads, followed by two rounds of homogenization and extraction of DNA from the resultant supernatant of the digest using SPRI beads. Rather than using 200uL of lysis buffer for tissue digestion as per the original protocol, our initial samples (e.g., small piece of dry blood spot for Alert and Mitivik Island samples, dried pellet containing approximately 10mg of packed red blood cells for Svalbard samples, and a grain-of-rice-sized skin tissue sample from Aleutian Islands and Pribilof Islands) were digested in 200uL of digestion buffer (100 mM NaCl, 50 mM Tris-HCl pH 8.0, 10 mM EDTA, 0.5% SDS) and 10uL of 20mg/mL proteinase K overnight at room temperature on a nutator. We did not include zirconia-silica beads for the homogenization step as per the original protocol considering our use of soft tissues which are comparatively easier to break

down. Other than that, we followed the published extraction protocol (Vo & Jedlicka, 2014) to extract DNA from the supernatant of our tissue digest. The genomic DNA was suspended in 50uL TE buffer and stored at -80°C until use.

RNA sample collection, extraction and sequencing

Sixteen snow buntings were chosen haphazardly for RNASeq from a pool of individuals housed at the avian facility of Université du Québec à Rimouski, QC, Canada. These individuals were captured near Rimouski, QC, Canada as wintering birds. All individuals used in the current study were humanely euthanized via cervical dislocation, their whole brain was collected and immediately preserved in a highly concentrated salt buffer (ammonium sulfate, 1 M sodium citrate, 0.5 M EDTA, H2SO4 to bring the pH to 5.2) for approximately fifteen minutes on ice until stored at -80°C. The sampling of the 16 individuals was equally spaced out from early January to the end of May 2018 to maximize mRNA expression diversity in the brain tissue samples.

Total RNA was extracted from brain tissue using TRIzol Reagents (Life Technologies, Mississauga, ON, Canada) according to the manufacturer's protocol. The RNA pellet was resuspended in Nuclease-Free Water (Thermo Fisher Scientific, Mississauga, ON, Canada) and RNA quality was assessed using the Eukaryotic RNA 6000 Nano assay on a 2100 Bioanalyzer (Agilent Technologies Canada Inc., Mississauga, ON, Canada). We ensured that all samples had RIN > 8.5 and a 28S/18S rRNA ratio > 0.8 when preparing the RNA-sequencing library for all sixteen birds. Final RNA aliquots were sent to the Genome Quebec Innovation Centre (McGill University, Montreal, QC, Canada) for 100bp paired-end sequencing in two lanes of an Illumina HiSeq4000 sequencer (Illumina Inc., San Diego, CA, USA).

RNA sequence analyses

Following sequencing, rRNA sequence reads were removed from the total raw sequence reads using SortMeRNA v2.1 (Kopyloca et al., 2012). Non-rRNA reads were then quality filtered using the default sliding window algorithm in Trimmomatic v0.38 (Bolger et al., 2014). This step allowed us to remove any low-quality sequences as well as adapter sequences added during RNA Sequencing library preparation. Following quality filtering, a *de-novo* transcriptome was assembled using fourteen out of sixteen samples (due to limitations on computational memory) using the default parameters with Trinity v2.8.4 (Hass et al., 2013) which included *in-silico* normalization for all reads. In the absence of a reference genome, and to ease the computational load for downstream data processing, the final reference transcriptome was assembled with only the longest isoform per transcript. Cleaned RNA sequence reads from all sixteen individuals were mapped to the final reference transcriptome using Burrow's Wheeler Alignment (BWA) v0.7.12 (Li & Durbin, 2009) (Appendix A1). Additionally, we assigned RG (Read Group) tags to all samples as unique sample IDs for each file. Resulting SAM files were converted to BAM files and sorted using SAMtools v1.3 (Li et al., 2009). We then removed PCR duplicates using Picard Tools (http://broadinstitute.github.io/picard) for each sample file. Lastly, the final BAM files were merged and low-quality mapping and supplemental alignments were removed with SAMtools v1.3 (Li et al., 2009).

SNP characterization and SNP marker development

The mapping information for all reads from the *de-novo* assembled reference transcriptome was used for nucleotide variant discovery using the Broad Institute's Genome Analysis Tools Kit (GATK) pipeline (DePristo et al., 2011; Van der Auwera et

al., 2013) to characterize and develop function gene locus SNPs. We performed quality recalibration, indel realignment and variant discovery on filtered-merged combined sequences, post-alignment, using GATK v4.1.7.0 (McKenna et al., 2010). Furthermore, we applied hard filtering parameters recommended for RNASeq experiments to detect variants (DePristo et al., 2011; Van der Auwera et al., 2013).

We used GeneMarkS-T (Besemer et al., 2001) to characterize open reading frames in our reference transcriptome and used SNPEff (Cingolani et al., 2012) to annotate variants and characterize them as missense, synonymous, upstream or downstream variants. We used the Trinotate pipeline (Bryant et al., 2017) to annotate all genes in our reference transcriptome and used LEMONS software (Levin et al., 2015) to predict intron splice junctions. It was important for us to identify the exon/intron boundaries to ensure that the SNP primers did not span introns since our goal was to use these primers to amplify genomic DNA.

By combining the SNPs (i.e., missense, synonymous, upstream or downstream variants) with gene annotation and predicted splice junction information, we were able to identify 11,378 useable SNPs (see Appendix A2). From those, we selected 192 SNP loci representing genes expected to be most likely to show local selection effects among our six populations. Broadly, the selected SNP loci were *a posteriori* placed in one of seven different functional categories: energetics, lipid metabolism, immune response, stress response, nervous system development, reproduction and cell-housekeeping processes (gene function categories for selected loci shown in Appendix A3, justifications for gene categories are shown in Appendix A4). We designed SNP primers to amplify a 100bp-150bp region surrounding the SNP of interest for the 192 loci using default settings with

Primer3 v4.1.0 (Untergasser et al., 2012). Forward and reverse universal adapters (ACCTGCCTGCC & ACGCCACCGAGC, respectively) were added to the 5' end of the designed primers to allow for the addition of sequencing adapters and sample-specific barcodes for High Throughput Sequencing (HTS). All primers were tested in 12.5uL reactions containing 20mM Tris-HCl pH 8.0, 10mM KCl, 10mM $(NH_4)_2SO_4$, 2mM $MgSO_4$, 0.1% Triton X-100, 0.1mg/mL bovine serum albumin (BSA), 200 µM of each dNTP, 200nM of forward and reverse primers, 0.5U of Taq polymerase (Bio Basic Canada Inc., Markham, ON, Canada), and 0.5uL of genomic DNA. The PCR cycling conditions were: 2 min at 95°C; 20s at 95°C, 20s at 58°C, 30s at 72°C (32 cycles); and 2 mins at 72°C. Of the 192 primer sets, 72 either did not amplify with genomic DNA, yielded non-specific amplification or produced an amplicon larger than 350bp: all of these were discarded from subsequent analyses. Details for the remaining 117 SNP primers are provided in Appendix A3 in Supplementary Data.

Microsatellite and SNP marker genotyping

Since our study included DNA extracted using two different methods, we first tested for DNA extraction method effects on the resulting genotypes. Five DNA samples selected at random from each of the 6 populations were extracted using both methods and genotyped at the candidate microsatellite and SNP loci (using detailed approaches noted below).

Microsatellite DNA marker data were first used to assess population genetic structure (which likely reflects variation in levels of reproductive isolation), and then they were used as the neutral controls for assessing divergence at the SNP loci. Specifically, the use of microsatellite markers allowed us to assess divergence at SNP loci relative to a putatively neutral microsatellite genetic divergence to highlight specific SNP loci that

may be under selection amongst the sampled populations. Briefly, all DNA samples were amplified at nine microsatellite loci with three PCR reactions: i) a first round of 20-cycle multiplex PCR (all primers combined) for preamplification of the DNA (this was done due to the small amount of DNA recovered from some samples) followed by ii) a second round of 30-cycle PCR with individual microsatellite primers, and iii) a final round of 5-cycle PCR to add fluorescent tags for fluorescence-based capillary electrophoresis. For each individual, we conducted the multiplex PCR in a 5uL reaction mixture containing 2.5uL of 2x Multiplex PCR Master mix (Qiagen Inc., Toronto, ON, Canada), 0.5uL of primer pool (10x primer mix containing 2uM each of all 9 primer pairs), and 1.0uL each of RNase-Free Water and template DNA. The amplification conditions were: 5 min at 95°C followed by 20 cycles of 30s at 95°C, 1 min 30s at 57°C, 30s at 72°C; and ending with 30 mins at 60°C. We diluted the PCR products 20-fold by adding 95uL of ddH_20. For the second round PCR, we amplified 2-4uL of the diluted multiplexed PCR product in a single-PCR reaction of 25uL which contained 10x Taq buffer (20mM Tris-HCl pH 8.0, 10mM KCl, 10 mM 10mM $(NH_4)_2SO_4$; Bio Basic Canada Inc., Markham, ON, Canada), 200uM each of dNTP, $MgSO_4$ (2uM), forward and reverse primers (2uM each), and 0.5U of Taq Polymerase (Bio Basic Canada Inc., Markham, ON, Canada). Thermocycling conditions were 95°C for 2 min; followed by 30 cycles of 95°C for 20s, locus-specific annealing temperature for 20s (56°C for CAM17, Lox8, Indigo29, SNBU682, and SNBU705; 58°C for Cuu28, POCC6, Ecit2, and CAM17), and 72°C for 30s, ending with 72°C for 2 min. For the final round of PCR, we used a PCR-based labelling technique where products from 1-4 loci were labelled with different dyes (6FAM, VIC, PET and NED; PCR conditions were identical to that of the second round

of PCR with the exception of 5 cycles instead of 30) and combined with Hi-Di formamide (Applied Biosystems, Foster City, CA, USA) and a GeneScan LIZ600 size standard (Applied Biosystems, Foster City, CA, USA) for separation on a SeqStudio Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Each sample was genotyped using GeneMapper software v3.5 and verified by eye.

We genotyped all individuals at the selected SNP loci using HTS. The HTS library preparation was completed using two rounds of PCR; multiplex followed by barcoding (ligation) PCR. We first amplified the 117 SNP loci using five separate multiplex PCRs for each sample (bird). Each multiplex PCR included 17-25 primer pairs (SNP locus groups shown in Appendix A3). Multiplex PCR used the Qiagen Multiplex PCR Plus Kit (Qiagen Inc., Toronto, ON, Canada). For each multiplex group, we first made 10x primer pools containing all primers within that group at equimolar concentration of 0.2uM. Each 7uL multiplex reaction contained 3.5uL Multiplex PCR Plus Master mix, 0.7uL of the 10x primer pool, 1.3uL ddH_2O, and 1.5uL genomic DNA. The amplification conditions were: 5 min at 95°C followed by 28 cycles of 30s at 95°C, 1 min 30s at 58°C and 30s at 72°C followed by 10 mins at 68°C. We diluted the multiplexed PCR product 10-fold with ddH_2O. Next, PCR products from each of the five multiplex reactions were pooled for each individual and cleaned using Sera-Mag Speed Beads (Cytiva, Mississauga, ON, Canada) to remove unincorporated dNTPs, primers, primer dimers and PCR buffers. We then ligated individual barcode sequences and HTS adaptor sequences to the PCR products in a second (ligation) short-cycle PCR. The 20uL PCR reaction included: 10x Taq buffer (20mM Tris-HCl pH 8.0, 10mM KCl, 10 mM 10mM $(NH_4)_2SO_4$; Bio Basic Canada Inc., Markham, ON, Canada), 2mM $MgSO_4$,

0.1mg/mL bovine serum albumin (BSA), 200uM of each dNTP, 200nM of forward and reverse primers, 0.5 U of Taq polymerase (Bio Basic Canada Inc., Markham, ON, Canada), and 10uL of pooled and cleaned multiplex PCR product. The PCR conditions for the ligation PCR were: 94°C for 2 min, followed by 6 cycles of 94°C for 30s, 60°C for 30s and 72°C for 60s, followed by 72°C for 5 min. This second PCR ligated a "barcode" sequence that allowed us to identify each sample for allocating sequence data to specific individuals post-sequencing. The barcoded products were pooled and gel-extracted using the GenCatch Gel Extraction Kit (Epoch Life Science Inc., Sugar Land, TX, USA) as per manufacturer's instructions. Purified pooled product was analyzed on an Agilent 2100 Bioanalyzer using a High Sensitivity chip (Agilent Technologies Canada Inc., Mississauga, ON, Canada) to verify the size and concentration of the library amplicons. Finally, the library was diluted to approximately 60pM and sequenced using Ion PGM Hi-Q chemistry in an Ion Chef System (Thermo Fisher Scientific Inc., Streetsville, ON, Canada). Specifically, the library was sequenced using an Ion 318 Chip Kit with an Ion PGM Sequencing 400 Kit (Thermo Fisher Scientific, Mississauga, ON).

Bioinformatics

After the HTS for the SNP loci, we used the FASTX Toolkit (Gordon & Hannon, 2010) and its Barcode Splitter script to demultiplex the sequences. We then trimmed off the sequencing adapters and barcodes from all reads using CUTADAPT v1.11 (Martin, 2011) and subsequently mapped the resulting PCR-amplified sequences to our reference transcriptome using BWA v0.7.12 (Li & Durbin, 2009) to identify the genes containing the amplified SNP regions. To genotype all individuals at target SNP loci, we used FreeBayes (Garrison & Marth, 2012), a Bayesian genetic variant detector. Since

FreeBayes detects many other variants such as small multi-nucleotide polymorphisms (MNPs), insertions and deletions (indels), composite insertions, and substitutions, we discarded such variants using VCFtools (Danecek et al. 2011) to ensure the presence of only the target SNPs in the resulting VCF file. Next, we further refined the VCF file by excluding the SNP locus markers that were called in less than 30% of individuals (16 of the SNPs out of 117 SNPs across all populations) and excluding individuals that were missing more than 10% of their genotypes (2 out of 221 individuals). Lastly, we only kept one SNP per amplicon (i.e., the original SNP used to design the primers for that amplicon) for further analyses to avoid any bias resulting from including multiple (linked) SNPs per amplicon.

Population genetic analyses

Testing for temporal effects

Since we had individuals collected across multiple years for most of our study populations, we first tested for temporal effects (i.e. a year effect) on allele frequencies. We conducted separate Fisher's exact tests of allele frequency variation for the microsatellite marker data for multi-year samples from Alert, Svalbard, Barrow and Mitivik Island using the genepop package (Rousset, 2008) in R v1.2.5 (R Core Team 2016). The Fisher's test results were corrected for multiple comparisons using the Bonferroni procedure (Rice, 1989) where needed. Since pre-correction p-values ranged from 0.08-0.50 for each population, we concluded that there were no temporal effects, hence we combined samples from multiple years for the Alert, Svalbard, Barrow and Mitivik Island populations.

Testing for Alaskan population neutral divergence

The Alaskan populations (Attu, Adak and Pribilof Islands) are geographically clustered (Figure 2.1), making it possible for individuals to migrate among the islands, and resulting in a single Alaskan metapopulation. We thus tested these three populations for neutral population divergence to allow combining the samples to obtain a more robust sample size for population genetic analyses. Based on the results of Fisher's test, we combined Attu and Adak Island samples from 1999 for further analysis, forming the population 'Aleutian Islands' since there were no significant differences in neutral allele frequencies ($p=0.14$). We retained Pribilof Islands individuals as a separate population for further analyses as it had significantly different ($p<0.00001$) neutral allele frequency distribution from the Aleutian Islands samples. These two Alaskan populations combined with the other four populations, resulted in a total of six populations for downstream analyses (Table 2.2).

Population genetic divergence

We assessed population differentiation across all sampled sites at neutral microsatellite and functional SNP markers using pairwise Fisher's exact test of allele frequency variation in the genepop package (Rousset, 2008) in R. We also estimated pairwise F_{ST} for both marker types using GENODIVE (version 3.0) (Miermans, 2020). We corrected all p-values for multiple comparisons using the sequential Bonferroni procedure (Rice, 1989) where necessary.

Neighbour-joining cluster analyses

To visually assess the pattern of population genetic divergence for the two marker types (microsatellite and SNP loci), we performed unrooted neighbour-joining cluster analyses

with Cavalli-Sforza and Edward's (1967) chord distance (D_c) using the 'ape' package (Paradis & Schliep, 2019) in R. Chord distance was used as it is expected to provide better tree topology estimation for closely related populations, although it may compromise branch length estimation (Angers & Bernatchez, 1998). We performed NJ cluster analyses for the two types of markers separately. The percent support for branches was estimated using bootstrapping, with replacement, among loci using 10,000 permutations in the 'poppr' package (Kamvar et al., 2014) in R.

Selection signatures at SNP loci

To detect a signature of selection at functional SNP loci, it is important to separate the effects of genetic drift from selection. For this purpose, we used the microsatellite markers to estimate the effects of genetic drift; it is expected that both functional SNP loci and microsatellites undergo genetic drift, but only SNP loci are expected to be under selection due to potential local habitat-specific environmental conditions.

Global selection at SNP loci

To assess whether SNP loci were under divergent selection across the six populations, we compared global estimates of Hedrick's G'_{ST} (Hedrick, 2005), calculated using the 'diveRsity' package (Keenan et al., 2013) in R, between neutral microsatellite and functional SNP loci. Hedrick's G'_{ST} is suitable for comparing genetic divergence measures among different marker types since it standardizes differences among markers for heterozygosity, allowing a comparison among loci with different levels of genetic variation (Hedrick, 2005). To assess an overall signature of selection at SNP loci, relative to microsatellite markers, across all populations, we first developed a 'neutral range' mean G'_{ST} with 99% confidence intervals (CI) for the nine microsatellite marker G'_{ST}

values using the 'diffCalc' function of the R package diveRsity. Specifically, we used bias-corrected bootstrapping across microsatellite loci to estimate the neutral CI range, representing neutrality expectation (presumably due to genetic drift) based on the G'_{ST} distribution of microsatellite markers. Next, we determined whether the G'_{ST} values for individual SNP loci fell outside of the neutral ranges, as such loci are likely to be under selection. Since the calculated neutral range for G'_{ST} did not include zero, we were able to identify SNP genes under stabilizing (lower than neutral expected) and divergent (higher than neutral expected) selection.

Pairwise selection at SNP loci

While it is possible for individual functional SNP markers to show a global selection signature, others may only show signatures of divergent or stabilizing selection at the pairwise population level due to specific differences in local conditions. To assess genetic divergence patterns among pairs of snow bunting populations, we calculated pairwise estimates of G'_{ST} using both microsatellite and SNP genotype data and compared the SNP loci pairwise G'_{ST} values with the presumed-neutral microsatellite loci range (created using 'diffCalc' function's bias-corrected bootstrapping loci approach as explained above) at the 99.9% CI to detect signatures of divergent and stabilizing selection. We used higher CI (99.9% versus 99% neutral CI used in global comparison) to avoid detection of false positives for pairwise comparisons since we are assessing 101 SNPs and fifteen population pairs. Corrections for multiple comparisons were not necessary as neutral range was individually developed for each comparison. We first combined all the results from the pairwise comparisons to investigate overall levels of genetic drift and selection, and also conducted a Chi-squared test to assess whether the

pattern of selection signatures differed across the seven gene function categories. However, for some population pairs it was not possible to identify SNPs under stabilizing selection since the neutral G'_{ST} range for that pairwise comparison included zero. As such, we have reported the SNP loci showing likely signals of divergent selection for all fifteen pairwise comparisons, but stabilizing selection for only nine of fifteen comparisons (i.e., we were unable to determine stabilizing selection in Alert/Barrow, Alert/Mitivik Island, Alert/Svalbard, Barrow/Mitivik Island, Barrow/Svalbard, and Mitivik Island/Svalbard comparisons). For the six comparisons which had neutral ranges that included zero, the SNP loci with G'_{ST} values less than expected neutral range (i.e., negative G'_{ST} values) were identified as "undetermined".

To gain further insight into specific genes that showed evidence for divergent selection, we explored the function of selected SNP loci with G'_{ST} values that had no "undetermined" classifications across any of the fifteen pairwise comparisons. Therefore, each SNP locus in this subset was either under genetic drift, stabilizing selection or divergent selection across all fifteen pairwise comparisons. This approach allowed us to assess the selection status of divergent SNP loci across all other population pairs – this allows the comparison of the role of these functional markers across all other population comparison(s) to highlight specific differences, allowing us to identify specific genes contributing to population divergence and local adaptation.

Selection signature and variant type

To characterize the role of SNP variant type (i.e., missense, synonymous, downstream or upstream), we determined the proportion of SNPs that showed signatures of genetic drift or selection for at the global and pairwise level (with combined data across all fifteen

comparisons) within each variant type. Given our functional SNPs are derived from transcribed sequences, we would expect selection to be more common among missense variants, as they would result in a different amino acid sequence in the protein.

Results

Microsatellite vs. SNP marker characteristics

We developed nine microsatellite markers (Appendix A5) and applied them across all individuals to assess reproductive isolation and establish "neutral" control data for functional SNP locus divergence. We also developed 117 functional SNP loci (Appendix A3) from a *de-novo* transcriptome for snow buntings which were expected to show local selection effects among breeding bunting populations based on the putative gene function. The microsatellite panel was more polymorphic than the SNP panel. The observed heterozygosity values for microsatellite markers were generally higher (0.345-0.708) than those of the SNP locus markers (0.098-0.111) (Appendix A6).

Sample collection and DNA extraction

Although the DNA from the tissue samples was extracted using two different methods, SPRI bead extractions and Qiagen kits, both methods yielded identical genotypes across all microsatellite and SNP markers, when tested using a subset of DNA samples from each of the six populations, we thus did not include extraction method as a covariate in our analyses. We were able to successfully extract DNA for all 221 samples across six populations for microsatellite and SNP marker genotyping.

RNA sequencing and SNP marker development

RNA sequencing produced more than 720.7 pair-end million reads from 16 samples (Appendix A1), 14 of which were used to assemble *de-novo* transcriptome containing 866.3Mb assembled into 534 815 trinity 'genes'. From this reference transcriptome, we used 373Mb of sequence data to assemble a novel transcriptome utilizing the longest isoform for each trinity gene. The resulting transcripts were used to characterize a total of 11,378 single nucleotide sequence variants using GATK, which is approximately 1 variant per 32.8Mbp of reference transcriptome. We first removed variants in transcripts with no valid start codon from the identified SNPs, as such variants are likely from incomplete or non-coding transcripts. This resulted in 9,756 useable sequence variants (see Appendix A2 for detailed summary statistics for SNP characterization). After optimization of multiplex groups, we retained 117 SNP loci (out of 192) to be genotyped in five multiplex groups (Appendix A3).

Microsatellite and SNP marker genotyping

We successfully genotyped all 221 individuals (across all six populations) at nine microsatellite loci. For SNP genotyping, 101 out of 117 SNP loci were genotyped in at least 70% of the individuals (our threshold for inclusion in the analyses). After genotyping, 219 out of the 221 individuals were successfully genotyped at >90% of the 101 SNP loci and were retained for population genetic analyses. Thus, all downstream population genetic analyses for the SNP loci were conducted using 101 SNP loci genotypes for 219 individuals. It should be noted that the final 101 SNPs consisted of 52 downstream, 11 upstream, 28 missense and 10 synonymous variants.

Population genetic analyses

Population genetic divergence

We performed pairwise analyses to characterize population differentiation using the neutral microsatellite and functional SNP locus marker data: Fisher's exact test and F_{ST} estimation. The microsatellite marker global F_{ST} value was 0.031 across all populations. The pairwise F_{ST} values across all fifteen comparisons ranged from -0.0001 to 0.100. The Fisher's exact test for microsatellite allele frequency distributions showed a highly significant population differentiation ($p \leq 0.001$) in all but one population pair (Barrow/Svalbard; $p=0.011$), that comparison was significant prior to Bonferroni correction (Table 2.3). The microsatellite marker pairwise F_{ST} values also showed highly significant population differentiation in 13/15 population pairs (F_{ST}: 0.009-0.100, $p \leq 0.012$) comparisons (Table 2.3). The population pairs Alert/Mitivik Island and Barrow/Svalbard (F_{ST}: -0.0001 for both pairs, pre-correction p-values of 0.564 and 0.464, respectively) did not show significant population differentiation before or after sequential Bonferroni correction (Table 2.3). Combined results from the Fisher's exact test and F_{ST} estimation at neutral markers provide evidence of partial reproductive isolation between all population pairs, with the exception of Barrow/Svalbard and Alert/Mitivik Island population pairs which exhibited weak isolation.

The SNP marker global F_{ST} value was 0.022 across all populations. The SNP marker pairwise F_{ST} values across all fifteen comparisons ranged from 0.004 to 0.053.The Fisher's exact test for functional SNP marker allele frequency distribution showed highly significant population differentiation in 9/15 population pairs ($p \leq 0.0009$), with non-significant differentiation for: Alert/Barrow, Alert/Mitivik Island,

Alert/Svalbard, Barrow/Mitivik Island, and Mitivik Island/Svalbard (pre-correction p-values: 0.56-0.88) (Table 2.4). The SNP marker pairwise F_{ST} estimates matched the Fisher's exact test results as the same population pairs (listed above) showed significant (F_{ST}: 0.024-0.053, $p \leq 0.003$) and non-significant (F_{ST}: 0.004-0.013, $p \geq 0.039$) differentiation (Table 2.4). Broadly, the combined results from the Fisher's exact test and F_{ST} estimation at the functional SNP markers did not show significant genetic differentiation between the non-migratory populations (Aleutian and Pribilof Islands), and among a majority (exception: Barrow/Svalbard comparison) of the migratory populations (Alert, Barrow, Mitivik Island, and Svalbard); however, all migratory - non-migratory population comparisons did show significant differentiation. The Barrow/Svalbard population pair had significant levels of SNP marker differentiation, although they were not significantly divergent based on neutral markers data. Overall, our analyses show substantial genetic divergence among our six sampled populations, indicative of at least partial reproductive isolation.

Neighbour-joining cluster diagrams

The neighbour-joining (NJ) cluster diagrams (Figure 2.2) based on microsatellite and SNP genotypes show similar overall patterns of divergence. The microsatellite marker data show three strongly supported clusters (100% branch support): Barrow & Svalbard, Alert & Mitivik Island, and Aleutian & Pribilof Islands; although Aleutian and Pribilof Islands also show strongly supported divergence (100% branch support) from each other (Figure 2.2a). On the other hand, the SNP genotype data do not show as strong support for population clustering among the six populations. However, the Aleutian & Pribilof Islands, and Barrow & Alert population pairs show strong patterns of divergence (99%

and 100% branch support) between the populations based on SNP marker data (Figure 2.2b).

Selection signatures at SNP loci

We assessed global and pairwise patterns of functional genetic divergence between six sampled breeding snow bunting populations. Specifically, we characterized patterns of divergence at the 101 SNP loci situated in transcribed regions in genes involved in energetics, lipid metabolism, immune response, stress response, nervous system development, reproduction and cell-housekeeping processes at a global (i.e. across all six populations) and pairwise (i.e. between all possible pairs of populations) level.

Global selection at SNP loci

The global G'_{ST} values for 9 microsatellite and 101 SNP loci across the six populations (221 individuals) were 0.203 and 0.0393, respectively. Per-locus G'_{ST} values ranged from 0.0454 to 0.535 for the microsatellite markers, and from -0.0789 to 0.267 for the SNP markers. Global differentiation patterns showed 94 out of 101 SNP loci to be consistent with stabilizing selection, as their level of divergence was lower than the neutral expectation (Figure 2.3; Appendix A7). The 7 remaining SNP loci showed divergence levels consistent with genetic drift (Figure 2.3; Appendix A7). The SNP loci showing a global genetic drift pattern of divergence belonged to four gene function categories: immune response (1 SNP); lipid metabolism (2 SNPs); nervous system development (1 SNP) and reproduction (3 SNPs). We did not detect any SNP loci showing a population divergence pattern consistent with divergent selection across the six populations, possibly due to differing patterns of divergent selection among the populations, making a pairwise analysis important to assess local divergence patterns.

Pairwise selection at SNP loci

To investigate population-level patterns in genetic divergence at the SNP loci, we calculated pairwise SNP marker G'_{ST} comparisons between all six populations. The overall pairwise G'_{ST} values ranged from 0.007 to 0.4508 for the microsatellite markers, and from 0.0076 to 0.0655 for the SNP locus markers across fifteen comparisons, depending on the population compared (Appendix A8).

To assess selection effects, we used a neutral expectation 99.9% CI (based on the microsatellite genotype data) to separate SNP loci likely evolving under genetic drift versus selection acting at the selected functional gene loci. Across all fifteen comparisons (1286 possible G'_{ST} values), we mostly observed signatures of stabilizing selection (51%) and genetic drift (38%), followed by undetermined (7%) and divergent selection (4%). We observed roughly equivalent patterns of divergence across the gene function categories (Appendix A9). The distribution of the types of selection did not differ significantly among different functional categories (χ^2 =20.33, p=0.32).

We were able to detect signatures of stabilizing selection in all but six population comparisons (Figure 2.4) where the neutral G'_{ST} ranges included zero (Alert/Barrow, Alert/Mitivik Island, Alert/Svalbard, Barrow/Mitivik Island, Barrow/Svalbard, and Mitivik Island/Svalbard). For these six comparisons, the SNP loci that had G'_{ST} values less than the neutral expected range (i.e., negative G'_{ST} values) were identified as "undetermined". Therefore, we are likely underestimating overall stabilizing selection effects. Overall, we observed 67.5%-96.3% of SNP loci under stabilizing selection among the nine population comparisons where we were able to test for signatures of stabilizing selection (Figure 2.4, Appendix A8).

We did not observe any signatures of divergent selection in six (Aleutian Islands/Alert, Aleutian Islands/Barrow, Aleutian Islands/Mitivik Island, Aleutian Islands/Pribilof Islands, Aleutian Islands/Svalbard, and Alert/Barrow) out of fifteen population comparisons (Figure 2.4). For the remaining nine population pairs, we observed 1.09%-23.1% of SNP loci under divergent selection (Figure 2.4, Appendix A8). The Barrow/Svalbard population comparison showed the most divergence (23.1%) based on our selected functional locus SNPs, followed by Pribilof Islands/Svalbard and Alert/Mitivik Island population comparisons which exhibited directional divergence at 7.23%, and 6.60% of tested SNP loci, respectively (Appendix A8).

To compare selection signatures across all pairwise population comparisons, SNP marker data would have to be available and the marker could not be classified as "undetermined" in any comparison. Thus, only a minority (11/101) of SNP marker loci could be broadly compared across all pairwise comparisons (Figure 2.5; Table 2.5). Based on those 11 SNP marker loci, high levels of stabilizing selection signatures were generally observed when the non-migratory populations (Aleutian & Pribilof Islands) were compared with other non-migratory or migratory populations (Alert, Barrow, Svalbard, and Mitivik Island), whereas comparisons between migratory populations showed mixed signatures of genetic drift and divergent selection depending on the SNP locus (Table 2.5). Of the 11 selected SNP loci, 7 were divergent in at least one population comparison, while the Barrow/Svalbard population pair comparison showed 6 of the 11 selected loci under divergent selection (Table 2.5). Broadly, the divergent genes from pairwise comparisons in this subset were associated with housekeeping, lipid metabolism, nervous system development, reproduction, and stress (Table 2.5, Appendix A10).

Discussion

Heterogeneous environmental conditions across time and space can drive adaptive population divergence among even partially reproductively isolated populations (Hereford, 2009). In this study, we assessed population structure and functional divergence among six geographically isolated breeding populations of Arctic-breeding snow buntings. Our neutral (microsatellite DNA loci) and functional (coding-gene SNPs) genetic marker data both show substantial population isolation among all populations, indicative of likely reproductive isolation. Furthermore, we demonstrated that the observed population differentiation patterns in the snow bunting populations we examined is a result of not only genetic drift, but stabilizing and divergent selection at functional genetic markers. The global divergence analyses showed strong evidence of stabilizing selection which is not surprising given the expected canalization of the vital functional gene loci chosen in this study. At the pairwise population comparison level, our functional marker results show signatures of both drift and selection, with functional divergent selection observed at some SNP loci. Such selection effects likely reflect the local adaptation of different snow bunting populations to their breeding grounds.

Genetic population structure

We demonstrated a greater spatial effect (i.e., among geographically dispersed populations) than temporal effect (i.e., among sample years within a population), likely due to the large geographical scale of sampling (pan-Arctic), but limited temporal sampling (one or two years per population). Although both of our marker types yielded broad spatial divergence patterns separating resident (Aleutian and Pribilof Islands) and migratory (Alert, Barrow, Mitivik Island, and Svalbard) populations, finer genetic

structure differed based on the marker type. As such, there was also finer population structure among migratory populations at neutral microsatellite markers as pairwise differentiation comparisons between migratory populations were significant, with Alert & Mitivik Island and Barrow & Svalbard being noticeably non-significant, indicative of substantial gene flow within each pair. The former pair had similar clustering pattern at the functional SNP loci, while the latter showed significant divergence likely due to strong selection despite geneflow. Our observation of gene flow between the Alert and Mitivik Island populations is new but supports previous work in this species using stable hydrogen isotope analysis and light-level geolocator tracking that suggested two parallel migratory systems divided by Hudson Bay as a migratory divide (Macdonald et al., 2012). Thus it is possible that the Alert population follows the same migratory route as the Mitivik Island population (i.e., to the West of Hudson bay, NU, Canada; Macdonald et al., 2012), and since the Mitivik island population has been shown through tracking studies to winter in the Canadian provinces of Alberta and Saskatchewan (Macdonald et al., 2012), it is further possible the individuals in these populations winter together, or even mix during migration to the breeding grounds. On the other hand, the presence of potential gene flow between Barrow & Svalbard was initially more surprising given the significant geographical distance between the two sample sites. Although we do not currently know where birds from the Barrow breeding population migrate to and over-winter, recent tracking work in the Svalbard population indicate they overwinter in the Asian Western Siberian Steppe where they utilize the high abundance of grain croplands and face very little interspecific competition (Snell et al., 2018). This could also be true for individuals breeding at Barrow, providing a potential mechanism for gene flow

between the two populations. If true, Svalbard birds would be migrating West in the Fall, and Barrow birds East in the fall, to potentially share wintering grounds in the Asian Western Siberian Steppes. Nevertheless, a detailed migration study is needed for Barrow snow buntings to empirically test the possibility of a shared use of wintering grounds.

While fairly spatially distant snow bunting populations showed genetic connectivity, we surprisingly found significant differentiation between the two non-migratory populations in Alaska (Aleutian and Pribilof Islands), based on microsatellite data. These populations exhibited substantial reproductive isolation likely due to their non-migratory life histories, despite being geographically close (Figure 2.1). Migratory life history is a critical component of genetic population structure; high migration rates result in genetically homogeneous populations, whereas restricted migration allows for development of genetically differentiated populations (Milgroom, 2015) due to high levels of reproductive isolation (Arguedas & Parker, 2000; Winker et al., 2000). Generally, the migratory behaviour of species has been a strong predictor of genetic diversity and differentiation (Arguedas & Parker, 2000; Tonteri et al., 2007). Long-distance migration can give rise to enhanced gene flow, due to errors in homing (i.e., straying behaviour) which can lead to low levels of reproductive isolation, hence low population divergence (Beacham & Withler, 2017; Bonin, 2021). However, individuals in non-migratory populations are not susceptible to homing errors, potentially leading to higher levels of divergence. Our results further support this idea since both marker types clustered resident and migratory populations separately. Overall, in addition to identifying significant global population differentiation, the genetic markers used in this study add to our knowledge of migratory connectivity patterns among breeding snow

bunting populations. More importantly, our results shed light on vulnerability of common wintering grounds for some populations should these sites face human-induced stressors such as habitat degradation.

Candidate gene approach to study local adaptation

While local adaptation is expected in reproductively isolated populations experiencing different environmental selection pressures, it has been rarely directly demonstrated empirically since it requires common-garden or reciprocal transplant experiments (Kawecki & Ebert, 2004) which are not practical for many wild populations (Blanquart et al., 2013). Studies in migratory bird species have identified patterns of variation in reproductive phenology such as migration and brood initiation (Wanamaker et al., 2020), morphological traits such as body size and weight (Blondel et al., 2006), as well as in traits involving song (Badyaev et al., 2008), personality (Mouchet et al., 2021), and plumage (Antoniazza et al., 2010) as locally adapted traits. Although those studies provide strong indirect evidence of local adaptation, they are not able to show a genetic component to the divergence, and hence the patterns reported may reflect phenotypic plasticity. In this study, we used a candidate gene approach to identify outlier loci under selection (stabilizing and divergent) across all sampled populations and between specific pairs of populations. Coupling the underlying function at the SNP gene loci under selection is a key step in determining likely environmental and ecological differences driving genetic variation among populations (Wellband et al., 2018). While more than a quarter (28/101) of the SNP markers represent coding missense variants, all were in very strong linkage disequilibrium with the target known-function genes. For this reason, our study differs from other genome-wide SNP approaches which investigate population-

level divergence using random SNPs located in both coding and non-coding regions of the genome (Tiffin & Ross-Ibarra, 2014; Pardo-Diez et al., 2015). While there are limitations with the use of a small panel (101 SNP loci) of candidate gene locus SNPs, our focussed selection of the candidate genes improves our power to detect patterns of population differentiation consistent with local adaptation in breeding snow bunting populations. Identification of patterns of local adaptation has implications for developing management and conservation plans that preserve locally important genetic diversity, especially as Arctic-migratory species continue to face strong effects of climate change worldwide.

Signatures of stabilizing and divergent selection

Generally, locally adapted populations are expected to exhibit gene polymorphism frequencies that evolve differently from the neutral model of evolution. Specifically, we expect genes demonstrating significantly higher (for divergent selection) or lower (for stabilizing selection) genetic differentiation than expected under neutral evolution models (Schlötterer, 2002; Hoban et al., 2016). Consistent with this idea, we found high levels of selection among our populations at functional locus markers. Only a handful of studies have assessed patterns of divergence at both coding (i.e. functional) and non-coding (i.e. presumed neutral) SNP marker loci, to interpret selection patterns in migratory bird species. Furthermore, the majority of those studies used randomly selected genome-wide SNPs where divergent selection is inferred as due to linkage disequilibrium with known or unknown genes. For example, Zhan et al. (2015) used a targeted approach when comparing thirteen wild populations of saker falcon (*Falco cherrug*) across Eurasia using 108 intronic SNPs and 36 exonic SNPs located in six known-function genes. In contrast

to their intronic SNPs, which did not show strong partitioning of individuals, 5 exonic SNPs within the *MHC* gene were under directional selection (F_{ST}>0.5), with the remaining candidate SNPs showed signatures of stabilizing selection or drift among saker falcon populations. Although SNP-based selection studies are becoming more common in migratory bird species (e.g., Ruegg et al., 2014; Bay et al., 2021; Larison et al., 2021; Ruegg et al., 2021), there have only been two such studies on Arctic-breeding migratory birds, both of which employed a non-targeted SNP selection approach and have reported no or low levels of selection. For example, Colston-Nepali et al. (2020) used restriction site-associated DNA sequencing (RAD-seq) to genotype six breeding colonies of northern fulmar (*Fulmarus glacialis*) at 6,614 genome-wide SNPs; however, no outlier loci were identified. A similar study by Tigano et al. (2017) analyzing 2220 genome-wide SNPs across five colonies of Arctic-breeding thick-billed murres revealed approximately 6% outlier SNPs and only 28% of those loci were under divergent selection, with the remaining loci under stabilizing selection. The non-targeted SNP scans across the genome in both is therefore less likely to detect high number of loci under selection than a coding-region only panel of SNPs. In fact, in Tigano et al.'s (2017) study, only 6 of the 111 identified outlier loci were successfully assigned gene function (i.e., GO terms), hence minimizing the functional relevance to the management or conservation of thick-billed murres. In contrast to the work on the northern fulmars and thick-billed murres, we observed high levels of selection across all sampled populations of snow buntings; with strong signatures of stabilizing selection at the global and pairwise levels, and with a few key SNP loci showing evidence of divergent selection in the pairwise comparisons. Our

results highlight the value of developing candidate gene SNP markers, despite the cost and complexity of transcriptome assembly for non-model species.

Our observations of high levels of stabilizing selection globally is consistent with the results of Tigano et al. (2017), and likely results from canalization of the functional SNP loci as they are involved in key organismal functions such as cellular housekeeping, immune function, reproduction, nervous system development, stress response, lipid metabolism and energetics. Additionally, our detection of broad patterns of stabilizing selection is plausible as sequence variants associated with critical function are expected to have similar allele frequencies across populations, lowering the overall levels of population differentiation at functional SNP loci. Since global analyses encompass an average effect at each locus across all populations, it is possible to observe an overall signal of stabilizing selection or genetic drift, yet specific differences at that locus may exist when pairs of populations are compared.

Spatially varying selection can promote local adaptation leading to site-specific adaptive polymorphisms (Tigano & Friesen, 2016). Although not all population pairs in our study exhibited signatures of divergent selection, we observed a range of 1-21 (~1-23%) SNP loci driving population differences in nine out of fifteen population pairs, suggesting the observed patterns of divergent selection are population specific. Curiously, we found relatively high levels of SNP loci under divergent selection in comparisons of Barrow and Svalbard (21 SNPs, 23.1%), and Alert and Mitivik Island (4 SNPs, 6.60%) population pairs, despite those population pairs exhibiting high gene flow based on neutral marker analyses. Generally, high levels of gene flow between populations decreases genetic divergence and therefore erodes the effects of local adaptation

(Lenormand, 2002; Blanquart et al., 2012; Aitken &Whitlock, 2013). However, our results indicate that the selective pressures are very strong for Barrow and Svalbard, and Alert and Mitivik Island population pairs, leading to divergent selection that overrides the effects of gene flow (Smith et al., 1997; Blondel et al., 1999). While divergent selection is important for local adaptation (and associated conservation considerations), the dominant selection signature across all pairwise population comparisons was stabilizing selection followed by genetic drift. Moreover, this observation was not driven by a specific functional category, as SNP loci under stabilizing selection belonged equally to all seven categories. Interestingly, pairwise comparisons of the migratory versus the non-migratory populations, as well as the comparison of the two non-migratory populations, revealed some of the highest levels of stabilizing selection (56-82 SNPs, ~68-96%). This is perhaps expected given our use of candidate genes involved in vital organismal function since variation at such loci can be highly maladaptive (Kawecki, 2000; Flatt, 2005), regardless of local habitat differences.

Genes of interest

Examining SNP loci that show recurring patterns of divergent selection can potentially identify gene functions that are important for local adaptation. For example, two missense variant SNP loci, Activin receptor type-2A (ACVR2A; SNP_41) and Receptor-type tyrosine-protein phosphatase zeta (PTPRZ1; SNP_60), showed divergent selection in more than one pairwise population comparison. While the functions of those two genes do not seem to be directly relevant for local adaptation in snow buntings, perhaps further exploration of these candidate loci in migratory birds in general may clarify their role in adaptive divergence. Among SNP loci with migratory life history relevance, ACVR2A

(divergent for Barrow and Mitivik, and Barrow and Svalbard population pairs) codes for a receptor that is involved in the induction of adipogenesis and growth (Donaldson et al., 1992) whereas PTPRZ1 (divergent for Pribilof Islands and Svalbard, and Barrow and Svalbard population pairs) is mainly involved in development of myelinating oligodendrocytes and is thought to play a role in the establishment of contextual memory and learning (The UniProt Consortium). It has been shown that fat reserves aid in thermoregulation (Vézina et al., 2012; Montgomerie & Lyon, 2020) and supress the adrenocortical response to environmental stress (Wingfield et al., 2004) allowing for successful breeding in harsh Arctic conditions in snow buntings. Although the importance of spatial memory and learning has not been studied in snow buntings, its importance is shown in other passerines in behaviours associated with food hoarding (Hitchcock & Sherry, 1990; Brodin, 1994; Healy & Krebs, 1996; Smulders & DeVoogd, 2000) and vocal communication (Nottebohm, 1999; Zeigler & Marler, 2004). It is also possible that genes of interest from this study reflect variation through linkage disequilibrium with other nearby genes which are under selection, therefore the selection effects at specific loci in this study should be interpreted with caution. Nevertheless, our results warrant further examination in snow buntings and possibly other Arctic-migratory avian species.

Conclusions and future directions

Arctic-breeding migratory bird species experience highly stochastic climate conditions resulting in a substantial variation in local abiotic parameters such as temperature, wind, precipitation and snow cover (Martin & Wiebe, 2004; Wingfield et al., 2004). These conditions are challenging for all Arctic species, but likely result in strong selective

pressures on Arctic-breeding birds due to short seasonal breeding times and the high energetic demands of migration and breeding (Le Pogam et al., 2021), ultimately leading to local adaptation of traits involved in survival and reproduction (Macdonald et al., 2012; Tigano et al., 2017; Snell et al., 2018). To our knowledge, this is the first study to investigate global population structure and patterns of genetic divergence consistent with local adaptation in a circum-polar Arctic-breeding bird. As predicted, we found significant divergence among the six breeding bunting populations which driven by both selection and drift. Consistent with our predictions, we observed strong levels of genetic drift and low levels of divergent selection at functional SNP loci; however, levels of stabilizing selection were high across breeding populations, which was inconsistent with our predictions. Identifying global population structure and patterns of genetic divergence is especially important for snow buntings and other Arctic-breeding migratory species as they face the strongest effects of climate change and therefore have to deal with high levels of variability during their critical breeding period (Walker et al., 2015). Changes in patterns of reproductive isolation over time can potentially result in a loss of fitness by altering the standing genetic variation in locally adapted populations. Therefore, the knowledge of functional divergence is crucial for identifying adaptive genotypes resilient against future stressors as it can add value to on-going monitoring and conservation of Arctic biodiversity.

References

Aitken, S. N., & Whitlock, M. C. (2013). Assisted gene flow to facilitate local adaptation to climate change. *Annual Review of Ecology, Evolution, and Systematics*, *44*, 367-388.

Allendorf, F. W. (2017). Genetics and the conservation of natural populations: allozymes to genomes. *Molecular Ecology*, *26*(2), 420-430.

Angers, B., & Bernatchez, L. (1998). Combined use of SMM and non-SMM methods to infer fine structure and evolutionary history of closely related brook charr (*Salvelinus fontinalis*, Salmonidae) populations from microsatellites. *Molecular Biology and Evolution, 15*(2), 143-159.

Antoniazza, S., Burri, R., Fumagalli, L., Goudet, J., & Roulin, A. (2010). Local adaptation maintains clinal variation in melanin-based coloration of European barn owls (*Tyto alba*). *Evolution: International Journal of Organic Evolution*, *64*(7), 1944-1954.

Arguedas, N., & Parker, P. G. (2000). Seasonal migration and genetic population structure in house wrens. *The Condor*, *102*(3), 517-528.

Atkins, K. E., & Travis, J. M. J. (2010). Local adaptation and the evolution of species' ranges under climate change. *Journal of Theoretical Biology*, 266(3), 449-457.

Badyaev, A. V., Young, R. L., Oh, K. P., & Addison, C. (2008). Evolution on a local scale: developmental, functional, and genetic bases of divergence in bill form and associated changes in song structure between adjacent habitats. *Evolution: International Journal of Organic Evolution*, *62*(8), 1951-1964.

Bay, R. A., Karp, D. S., Saracco, J. F., Anderegg, W. R., Frishkoff, L. O., Wiedenfeld, D., ... & Ruegg, K. (2021). Genetic variation reveals individual-level climate tracking across the annual cycle of a migratory bird. *Ecology Letters*, *24*(4), 819-828.

Beacham, T. D., & Withler, R. E. (2017). Population structure of sea-type and lake-type sockeye salmon and kokanee in the Fraser River and Columbia River drainages. *PLoS One*, *12*(9), e0183713.

Beaumont, M. A., & Balding, D. J. (2004). Identifying adaptive genetic divergence among populations from genome scans. *Molecular Ecology*, *13*(4), 969-980.

Bensch, S., Price, T., & Kohn, J. (1997). Isolation and characterization of microsatellite loci in a *Phylloscopus* warbler. *Molecular Ecology*, *6*(1), 91-92.

Besemer, J., Lomsadze, A., & Borodovsky, M. (2001). GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. *Nucleic Acids Research*, *29*(12), 2607-2618.

Bhargava, A., & Fuentes, F. F. (2010). Mutational dynamics of microsatellites. *Molecular Biotechnology*, *44*(3), 250-266.

Blanquart, F., Gandon, S., & Nuismer, S. L. (2012). The effects of migration and drift on local adaptation to a heterogeneous environment. *Journal of Evolutionary Biology*, *25*(7), 1351-1363.

Blanquart, F., Kaltz, O. Nuismer, S. L., & Gandon S. (2013). A practical guide to measuring local adaptation. *Ecology Letters*, *16*(9), 1195-1205.

Blondel, J., Dias, P. C., Perret, P., Maistre, M., & Lambrechts, M. M. (1999). Selection-based biodiversity at a small spatial scale in a low-dispersing insular bird. *Science*, *285*(5432), 1399-1402.

Blondel, J., Thomas, D. W., Charmantier, A., Perret, P., Bourgault, P., & Lambrechts, M. M. (2006). A thirty-year study of phenotypic and genetic variation of blue tits in Mediterranean habitat mosaics. *Bioscience*, *56*(8), 661-673.

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114-2120.

Bonin, C. A. (2021). Genetic Consequences of Dispersal, Philopatry and Reproductive Behaviors. In *Ethology and Behavioral Ecology of Otariids and the Odobenid* (pp. 223-241). Springer, Cham.

Both, C. (2010). Food availability, mistiming, and climatic change. *Effects of climate change on birds* (pp. 129-147). Oxford University Press.

Both, C., Bouwhuis, S., Lessells, C. M., & Visser, M. E. (2006). Climate change and population declines in a long-distance migratory bird. *Nature*, *441*(7089), 81-83.

Branch, C. L., & Pravosudov, V. V. (2015). Mountain chickadees from different elevations sing different songs: acoustic adaptation, temporal drift or signal of local adaptation?. *Royal Society Open Science*, *2*(4), 150019.

Brodin, A. (1994). The disappearance of caches that have been stored by naturally foraging willow tits. *Animal Behaviour*, *47*(3), 730-732.

Bryant, D. M., Johnson, K., DiTommaso, T., Tickle, T., Couger, M. B., Payzin-Dogru, D., ... & Bateman, J. (2017). A tissue-mapped axolotl de novo transcriptome enables identification of limb regeneration factors. *Cell Reports*, *18*(3), 762-776.

Butcher, G. S., & Niven, D. K. (2007). Combining data from the Christmas Bird Count and the Breeding Bird Survey to determine the continental status and trends of North America birds.

Cingolani, P., Platts, A., Wang, L. L., Coon, M., Nguyen, T., Wang, L., ... & Ruden, D. M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly*, *6*(2), 80-92.

Colston-Nepali, L., Provencher, J. F., Mallory, M. L., Franckowiak, R. P., Sun, Z., Robertson, G. J., & Friesen, V. L. (2020). Using genomic tools to inform management of the Atlantic northern fulmar. *Conservation Genetics*, *21*(6), 1037-1050.

Cotton, P. A. (2003). Avian migration phenology and global climate change. *Proceedings of the National Academy of Sciences*, *100*(21), 12219-12222.

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., ... & McVean, G. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156-2158.

Dawson, D. A., Horsburgh, G. J., Küpper, C., Stewart, I. R., Ball, A. D., Durrant, K. L., ... & Krupa, A. P. (2010). New methods to identify conserved microsatellite loci and develop primer sets of high cross-species utility–as demonstrated for birds. *Molecular Ecology Resources*, *10*(3), 475-494.

DePristo, M. A., Banks, E., Poplin, R., Garimella, K. V., Maguire, J. R., Hartl, C., ... & Daly, M. J. (2011). A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nature Genetics*, *43*(5), 491-498.

Donaldson, C. J., Mathews, L. S., & Vale, W. W. (1992). Molecular cloning and binding properties of the human type II activin receptor. *Biochemical and Biophysical Research Communications*, *184*(1), 310-316.

Ellegren, H. (2000). Microsatellite mutations in the germline:: implications for evolutionary inference. *Trends in Genetics*, *16*(12), 551-558.

Falconer, C. M., Mallory, M. L., & Nol, E. (2008). Breeding biology and provisioning of nestling snow buntings in the Canadian High Arctic. *Polar Biology*, *31*(4), 483-489.

Ferguson, L., Lee, S. F., Chamberlain, N., Nadeau, N., Joron, M., Baxter, S., ... & Jiggins, C. (2010). Characterization of a hotspot for mimicry: assembly of a butterfly wing transcriptome to genomic sequence at the HmYb/Sb locus. *Molecular Ecology*, *19*, 240-254.

Flatt, T. (2005). The evolutionary genetics of canalization. *The Quarterly Review of Biology*, *80*(3), 287-316.

Fossøy, F., Stokke, B. G., Kåsi, T. K., Dyrset, K., Espmark, Y., Hoset, K. S., ... & Moksnes, A. (2014). Reproductive success is strongly related to local and regional climate in the Arctic snow bunting (*Plectrophenax nivalis*). *Polar Biology*, *38*(3), 393-400.

Fraser, D. J., & Bernatchez, L. (2001). Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Molecular Ecology*, *10*(12), 2741-2752.

Fraser, D. J., Weir, L. K., Bernatchez, L., Hansen, M. M., & Taylor, E. B. (2011). Extent and scale of local adaptation in salmonid fishes: review and meta-analysis. *Heredity*, *106*(3), 404-420.

Galindo, J., Grahame, J. W., & Butlin, R. K. (2010). An EST-based genome scan using 454 sequencing in the marine snail *Littorina saxatilis*. *Journal of Evolutionary Biology*, *23*(9), 2004-2016.

Garrison, E., & Marth, G. (2012). Haplotype-based variant detection from short-read sequencing. *arXiv preprint arXiv:1207.3907*.

Gibbs, H. L., Tabak, L. M., & Hobson, K. (1999). Characterization of microsatellite DNA loci for a neotropical migrant songbird, the Swainson's thrush (*Catharus ustulatus*). *Molecular Ecology*, *8*, 1551-1551.

Gordo, O. (2007). Why are bird migration dates shifting? A review of weather and climate effects on avian migratory phenology. *Climate Research*, *35*(1-2), 37-58.

Gordon, A., & Hannon, G. J. (2010). Fastx-toolkit. FASTQ/A short-reads preprocessing tools (unpublished), http://hannonlab.cshl.edu/fastx_toolkit.

Gorter, F. A., Scanlan, P. D., & Buckling, A. (2016). Adaptation to abiotic conditions drives local adaptation in bacteria and viruses coevolving in heterogeneous environments. *Biology Letters*, *12*(2), 20150879.

Graham, B. A., Sandoval, L., Dabelsteen, T., & Mennill D. J. (2016). A test of the Acoustic Adaptation Hypothesis in three types of tropical forest: degradation of male and female Rufous-and-white Wren songs. *Bioacoustics*, *26*(1), 37-61.

Guindre-Parker, S., Gilchrist, H. G., Baldo, S., Doucet, S. M., & Love, O. P. (2013a). Multiple achromatic plumage ornaments signal to multiple receivers. *Behavioral Ecology*, *24*(3), 672-682.

Guindre-Parker, S., Gilchrist, H. G., Baldo, S., & Love, O. P. (2013b). Alula size signals male condition and predicts reproductive performance in an Arctic-breeding passerine. *Journal of Avian Biology*, *44*(3), 209-215.

Gullett, P., Hatchwell, B. J., Robinson, R. A., & Evans, K. L. (2013). Phenological indices of avian reproduction: cryptic shifts and prediction across large spatial and temporal scales. *Ecology and Evolution*, *3*(7), 1864-1877.

Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P. D., Bowden, J., ... & MacManes, M. D. (2013). De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature Protocols*, *8*(8), 1494-1512.

Healy, S. D., & Krebs, J. R. (1996). Food storing and the hippocampus in Paridae. *Brain, Behavior and Evolution*, *47*(4), 195-199.

Hedrick, P. W. (2005). A standardized genetic differentiation measure. *Evolution*, *59*(8), 1633-1638.

Hereford, J. (2009). A quantitative survey of local adaptation and fitness trade-offs. *The American Naturalist*, *173*(5), 579-588.

Hitchcock, C. L., & Sherry, D. F. (1990). Long-term memory for cache sites in the black-capped chickadee. *Animal Behaviour*, *40*(4), 701-712.

Hoban, S., Kelley, J. L., Lotterhos, K. E., Antolin, M. F., Bradburd, G., Lowry, D. B., ... & Whitlock, M. C. (2016). Finding the genomic basis of local adaptation: pitfalls, practical solutions, and future directions. *The American Naturalist*, *188*(4), 379-397.

Hoset, K. S., Espmark, Y., Fossøy, F., Stokke, B. G., Jensen, H., Wedege, M. I., & Moksnes, A. (2014). Extra-pair paternity in relation to regional and local climate in an Arctic-breeding passerine. *Polar Biology*, *37*(1), 89-97.

Job, J. R., Kohler, S. L., & Gill, S. A. (2015). Song adjustments by an open habitat bird to anthropogenic noise, urban structure, and vegetation. *Behavioural Ecology*, *27*(6), 1734-1744.

Jonzén, N., Lindén, A., Ergon, T., Knudsen, E., Vik, J. O., Rubolini, D., ... & Stenseth, N. C. (2006). Rapid advance of spring arrival dates in long-distance migratory birds. *Science*, *312*(5782), 1959-1961.

Jump, A. S., & Penuelas, J. (2005). Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology Letters*, *8*(9), 1010-1020.

Jump, A. S., Marchant, R., & Peñuelas, J. (2009). Environmental change and the option value of genetic diversity. *Trends in Plant Science*, *14*(1), 51-58.

Kamvar, Z. N., Tabima, J. F., & Grünwald, N. J. (2014). Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ*, *2*, e281. https://doi.org/10.7717/peerj.281.

Kawecki, T. J. (2000). The evolution of genetic canalization under fluctuating selection. *Evolution*, *54*(1), 1-12.

Kawecki, T. J., & Ebert D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, *7*(12), 1225-1241.

Keenan, K., McGinnity, P., Cross, T. F., Crozier, W. W., & Prodöhl, P. A. (2013). diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods in Ecology and Evolution*, *4*(8), 782-788.

Kirk, H., & Freeland, J. R. (2011). Applications and implications of neutral versus non-neutral markers in molecular ecology. *International Journal of Molecular Sciences*, *12*(6), 3966-3988.

Kopylova, E., Noé, L., & Touzet, H. (2012). SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. *Bioinformatics*, *28*(24), 3211-3217.

Kuhn, K., Schwenk, K., Both, C., Canal, D., Johansson, U. S., van der Mije, S., ... & Päckert, M. (2013). Differentiation in neutral genes and a candidate gene in the pied flycatcher: using biological archives to track global climate change. *Ecology and Evolution*, *3*(14), 4799-4814.

Lambrechts, M. M., Perret, P., & Blondel, J. (1996). Adaptive differences in the timing of egg laying between different populations of birds result from variation in photoresponsiveness. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *263*(1366), 19-22.

Larison, B., Lindsay, A. R., Bossu, C., Sorenson, M. D., Kaplan, J. D., Evers, D. C., ... & Ruegg, K. (2021). Leveraging genomics to understand threats to migratory birds. *Evolutionary Applications*, *14*(6), 1646-1658.

Lehtonen, P. K., Laaksonen, T., Artemyev, A. V., Belskii, E., Berg, P. R., Both, C., ... & Primmer, C. R. (2012). Candidate genes for colour and vision exhibit signals of selection across the pied flycatcher (*Ficedula hypoleuca*) breeding range. *Heredity*, *108*(4), 431-440.

Leimu, R., & Fischer, M. (2008). A meta-analysis of local adaptation in plants. *PloS one*, *3*(12), e4010.

Lenormand, T. (2002). Gene flow and the limits to natural selection. *Trends in Ecology & Evolution*, *17*(4), 183-189.

Le Pogam, A., O'Connor, R. S., Love, O. P., Petit, M., Régimbald, L., & Vézina, F. (2021). Coping with the worst of both worlds: Phenotypic adjustments for cold acclimatization benefit northward migration and arrival in the cold in an Arctic-breeding songbird. *Functional Ecology*, *35*(6), 1240-1254.

Levin, L., Bar-Yaacov, D., Bouskila, A., Chorev, M., Carmel, L., & Mishmar, D. (2015). LEMONS–a tool for the identification of splice junctions in transcriptomes of organisms lacking reference genomes. *PloS one*, *10*(11), e0143329.

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics*, *25*(14), 1754-1760.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., ... & Durbin, R. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics*, *25*(16), 2078-2079.

Luikart, G., England, P. R., Tallmon, D., Jordan, S., & Taberlet, P. (2003). The power and promise of population genomics: from genotyping to genome typing. *Nature Reviews Genetics*, *4*(12), 981-994.

Lyngs, P. (2003). *Migration and Winter Ranges of Birds in Greenland*. Copenhagen: Danish Ornithological Society.

Macdonald, C. A., Fraser, K. C., Gilchrist, H. G., Kyser, T. K., Fox, J. W., & Love, O. P. (2012). Strong migratory connectivity in a declining Arctic passerine. *Animal Migration*, *1*(1), 23-30.

Martin, K., & Wiebe, K. L. (2004). Coping mechanisms of alpine and arctic breeding birds: extreme weather and limitations to reproductive resilience. *Integrative and Comparative Biology*, *44*(2), 177-185.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. Journal*, *17*(1), 10-12.

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., ... & DePristo, M. A. (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research*, *20*(9), 1297-1303.

McKinnon, E. A., Macdonald, C. M., Gilchrist, H. G., & Love, O. P. (2016). Spring and fall migration phenology of an Arctic-breeding passerine. *Journal of Ornithology*, *157*(3), 681-693.

Meirmans, P. G. (2020). genodive version 3.0: Easy-to-use software for the analysis of genetic data of diploids and polyploids. *Molecular Ecology Resources*, *20*(4), 1126-1131.

Milgroom, M. G. (2015). Migration and population structure. *Population biology of plant pathogens: Genetics, Ecology, and Evolution*, 119-146.

Montgomerie, R. and B. Lyon (2020). Snow Bunting (*Plectrophenax nivalis*), version 1.0. In Birds of the World (S. M. Billerman, B. K. Keeney, P. G. Rodewald, and T. S. Schulenberg, Editors). Cornell Lab of Ornithology, Ithaca, NY, USA. https://doi-org.ledproxy2.uwindsor.ca/10.2173/bow.snobun.01

Mouchet, A., Cole, E. F., Matthysen, E., Nicolaus, M., Quinn, J. L., Roth, A. M., ... & Dingemanse, N. J. (2021). Heterogeneous selection on exploration behavior within and among West European populations of a passerine bird. *Proceedings of the National Academy of Sciences*, *118*(28), 1-6.

Nottebohm, F. (1999). The anatomy and timing of vocal learning in birds. *The Design of Animal Communication* (pp. 63-110). Massachusetts Institute of Technology.

O'Connor, R. S., Le Pogam, A., Young, K. G., Robitaille, F., Choy, E. S., Love, O. P., ... & Vézina, F. (2021). Limited heat tolerance in an Arctic passerine: Thermoregulatory implications for cold-specialized birds in a rapidly warming world. *Ecology and Evolution*, *11*(4), 1609-1619.

Ouborg, N. J., Pertoldi, C., Loeschcke, V., Bijlsma, R. K., & Hedrick, P. W. (2010). Conservation genetics in transition to conservation genomics. *Trends in Genetics*, *26*(4), 177-187.

Paradis, E., & Schliep, K. (2019). ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, *35*(3), 526-528

Pardo-Diaz, C., Salazar, C., & Jiggins, C. D. (2015). Towards the identification of the loci of adaptive evolution. *Methods in Ecology and Evolution*, *6*(4), 445-464.

Petren, K. (1998). Microsatellite primers from Geospiza fortis and cross-species amplification in Darwin's finches. *Molecular Ecology*, *7*(12), 1782-1784.

Piertney, S. B., Marquiss, M., & Summers, R. (1998). Characterization of tetranucleotide microsatellite markers in the Scottish crossbill (*Loxia scotica*). *Molecular Ecology*, *7*(9), 1261-1263.

Price, R. M., Andrews, T. C., McElhinny, T. L., Mead, L. S., Abraham, J. K., Thanukos, A., & Perez, K. E. (2014). The genetic drift inventory: a tool for measuring what advanced undergraduates have mastered about genetic drift. *CBE—Life Sciences Education*, *13*(1), 65-75.

Renaut, S., Nolte, A. W., & Bernatchez, L. (2010). Mining transcriptome sequences towards identifying adaptive single nucleotide polymorphisms in lake whitefish species pairs (Coregonus spp. Salmonidae). *Molecular Ecology*, *19*(s1), 115-131.

Reuter, J. A., Spacek, D. V., & Snyder, M. P. (2015). High-throughput sequencing technologies. *Molecular Cell*, *58*(4), 586-597.

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution*, *43*(1), 223-225.

Ridgeway, R. (1887). A Manual of North American Birds. J.B Lippincott Company.

Rolland, J., Jiguet, F., Jønsson, K. A., Condamine, F. L., & Morlon, H. (2014). Settling down of seasonal migrants promotes bird diversification. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1784), 20140473.

Romero, L. M., Soma, K. K., & Wingfield, J. C. (1998). Changes in pituitary and adrenal sensitivities allow the snow bunting (*Plectrophenax nivalis*), an Arctic-breeding song bird, to modulate corticosterone release seasonally. *Journal of Comparative Physiology B*, *168*(5), 353-358.

Rousset, F. (2008). genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources*, *8*(1), 103-106.

Ruegg, K. C., Anderson, E. C., Paxton, K. L., Apkenas, V., Lao, S., Siegel, R. B., ... & Smith, T. B. (2014). Mapping migration in a songbird using high-resolution genetic markers. *Molecular Ecology*, *23*(23), 5726-5739.

Ruegg, K., Anderson, E. C., Somveille, M., Bay, R. A., Whitfield, M., Paxton, E. H., & Smith, T. B. (2021). Linking climate niches across seasons to assess population vulnerability in a migratory bird. *Global Change Biology*, *27*(5), 3519-3531.

Schlötterer, C. (2002). Towards a molecular characterization of adaptation in local populations. *Current Opinion in Genetics & Development*, *12*(6), 683-687.

Sefc, K. M., Payne, R. B., & Sorenson, M. D. (2001). Characterization of microsatellite loci in village indigobirds *Vidua chalybeata* and cross-species amplification in estrildid and ploceid finches. *Molecular Ecology Notes*, *1*(4), 252-254.

Sekercioglu, C. H. (2007). Conservation ecology: area trumps mobility in fragment bird extinctions. *Current Biology*, *17*(8), R283-R286.

Slabbekoorn, H., & Smith, T. B. (2002). Bird song, ecology and speciation. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *357*(1420), 493-503.

Smith, T. B., Wayne, R. K., Girman, D. J., & Bruford, M. W. (1997). A role for ecotones in generating rainforest biodiversity. *Science*, *276*(5320), 1855-1857.

Smulders, T. V., & DeVoogd, T. J. (2000). Expression of immediate early genes in the hippocampal formation of the black-capped chickadee (*Poecile atricapillus*) during a food-hoarding task. *Behavioural Brain Research*, *114*(1-2), 39-49.

Snell, K. R., Stokke, B. G., Moksnes, A., Thorup, K., & Fossøy, F. (2018). From Svalbard to Siberia: Passerines breeding in the High Arctic also endure the extreme cold of the Western Steppe. *PloS one*, *13*(9), e0202114.

Tiffin, P., & Ross-Ibarra, J. (2014). Advances and limits of using population genetics to understand local adaptation. *Trends in Ecology & Evolution*, *29*(12), 673-680.

Tigano, A., & Friesen, V. L. (2016). Genomics of local adaptation with gene flow. *Molecular Ecology*, *25*(10), 2144-2164.

Tigano, A., Shultz, A. J., Edwards, S. V., Robertson, G. J., & Friesen, V. L. (2017). Outlier analyses to test for local adaptation to breeding grounds in a migratory arctic seabird. *Ecology and Evolution*, *7*(7), 2370-2381.

Toews, D. P., Campagna, L., Taylor, S. A., Balakrishnan, C. N., Baldassarre, D. T., Deane-Coe, P. E., ... & Winger, B. M. (2016). Genomic approaches to understanding population divergence and speciation in birds. *The Auk: Ornithological Advances*, *133*(1), 13-30.

Tonteri, A., Veselov, A. J., Titov, S., Lumme, J., & Primmer, C. R. (2007). The effect of migratory behaviour on genetic diversity and population divergence: a comparison of anadromous and freshwater Atlantic salmon *Salmo salar*. *Journal of Fish Biology*, *70*, 381-398.

Turner, T. L., Bourne, E. C., Von Wettberg, E. J., Hu, T. T., & Nuzhdin, S. V. (2010). Population resequencing reveals local adaptation of *Arabidopsis lyrata* to serpentine soils. *Nature Genetics*, *42*(3), 260-263.

UniProt Consortium. (2015). UniProt: a hub for protein information. *Nucleic Acids Research*, *43*(D1), D204-D212.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3—new capabilities and interfaces. *Nucleic Acids Research*, *40*(15), e115-e115.

Valladares, F., Matesanz, S., Guihaumon, F., Araújo, M. D., Balaguer, L., Benito-Garzón, M., … & Zavala, M. A. (2014). The effects of phenotypic plasticity and local adatation on forecasts of species range shifts under climate change. *Ecology Letters*, 17(11), 1351-1364.

Van der Auwera, G. A., Carneiro, M. O., Hartl, C., Poplin, R., Del Angel, G., Levy-Moonshine, A., ... & Banks, E. (2013). From FastQ data to high-confidence variant calls: the genome analysis toolkit best practices pipeline. *Current Protocols in Bioinformatics*, *43*(1), 11-10.

Van der Auwera, G. A., Carneiro, M. O., Hartl, C., Poplin, R., Del Angel, G., Levy-Moonshine, A., ... & DePristo, M. A. (2013). From FastQ data to high-confidence variant calls: the genome analysis toolkit best practices pipeline. *Current Protocols in Bioinformatics*, *43*(1), 11-10.

Vézina, F., Williams, T. D., Piersma, T., & Guy Morrison, R. I. (2012). Phenotypic compromises in a long-distance migrant during the transition from migration to reproduction in the High Arctic. *Functional Ecology*, *26*(2), 500-512.

Visser, M. E., Gienapp, P., Husby, A., Morrisey, M., de la Hera, I., Pulido, F., & Both, C. (2015). Effects of spring temperatures on the strength of selection on timing of reproduction in a long-distance migratory bird. *PLoS Biology*, *13*(4), e1002120.

Vo, A. T., & Jedlicka, J. A. (2014). Protocols for metagenomic DNA extraction and Illumina amplicon library preparation for faecal and swab samples. *Molecular Ecology Resources*, *14*(6), 1183-1197.

Walker, B. G., Meddle, S. L., Romero, L. M., Landys, M. M., Reneerkens, J., & Wingfield, J. C. (2015). Breeding on the extreme edge: modulation of the adrenocortical response to acute stress in two High Arctic passerines. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *323*(4), 266-275.

Wan, Q. H., Wu, H., Fujihara, T., & Fang, S. G. (2004). Which genetic marker for which conservation genetics issue?. *Electrophoresis*, *25*(14), 2165-2176.

Wanamaker, S. M., Singh, D., Byrd, A. J., Smiley, T. M., & Ketterson, E. D. (2020). Local adaptation from afar: migratory bird populations diverge in the initiation of reproductive timing while wintering in sympatry. *Biology Letters*, *16*(10), 20200493.

Warner, N. A., Sagerup, K., Kristoffersen, S., Herzke, D., Gabrielsen, G. W., & Jenssen, B. M. (2019). Snow buntings (*Plectrophenax nivalis*) as bio-indicators for exposure differences to legacy and emerging persistent organic pollutants from the Arctic terrestrial environment on Svalbard. *Science of The Total Environment*, *667*, 638-647.

Wellband, K. W., Pettitt-Wade, H., Fisk, A. T., & Heath, D. D. (2018). Standing genetic diversity and selection at functional gene loci are associated with differential invasion success in two non-native fish species. *Molecular Ecology*, *27*(7), 1572-1585.

Wingfield, J. C., Owen-Ashley, N. T., Benowitz-Fredericks, Z. M., Lynn, S., Hahn, T. P., Wada, H., ... & Romero, L. M. (2004). Arctic spring: the arrival biology of migrant birds. *Acta Zoologica Sinica*, *50*(6), 948-960.

Winker, K., Graves, G. R., & Braun, M. J. (2000). Genetic differentiation among populations of a migratory songbird: *Limnothlypis swainsonii*. *Journal of Avian Biology*, *31*(3), 319-328.

Wonke, G., Wallschläger, D., Moll, K., & Tiedemann, R. (2007). Ten new microsatellite loci for the yellowhammer (*Emberiza citrinella*) and their cross-species applicability among related taxa. *Molecular Ecology Notes*, *7*(6), 1278-1280.

Yeaman, S., & Otto, S. (2011). Establishment and maintenance of adaptive genetic divergence under migration, selection and drift. *Evolution*, 65(7), 2123-2129.

Zeigler, H., & Marler, P. E. (2004). Behavioral neurobiology of birdsong. New York Academy of Sciences.

Zhan, X., Dixon, A., Batbayar, N., Bragin, E., Ayas, Z., Deutschova, L., ... & Bruford, M. W. (2015). Exonic versus intronic SNPs: contrasting roles in revealing the population genetic differentiation of a widespread bird species. *Heredity*, *114*(1), 1-9.

Zimmerman, S. J., Aldridge, C. L., Oh, K. P., Cornman, R. S., & Oyler-McCance, S. J. (2019). Signatures of adaptive divergence among populations of an avian species of conservation concern. *Evolutionary Applications*, *12*(8), 1661-1677.

Tables

Table 2.1: Location and life history trait data for six DNA-sampled snow bunting (*Plectrophenax nivalis*) populations used in the population genetic study.

	Alert, NU, Canada (A)	**Barrow, AK, USA (B)**	**Mitivik Island, NU, Canada (M)**	**Svalbard, Norway (S)**	**Aleutian Islands, AK, USA (AI)**	**Pribilof Islands, AK, USA (PI)**
Sub-species [1]	*Plectrophenax nivalis nivalis*	*Plectrophenax nivalis nivalis*	*Plectrophenax nivalis nivalis*	*Plectrophenax nivalis nivalis*	*Plectrophenax nivalis townsendi*	*Plectrophenax nivalis townsendi*
Migratory/ Resident	Migratory [1]	Migratory [1]	Migratory [2]	Migratory [3]	Resident **[1]**	Resident **[1]**
Migration Distance	Currently unknown	Currently unknown	Fall: ~2660 ± 59 km; Spring: 2147 ± 69 km [4]	Fall: >1000 km [5]	N/A	N/A
Nesting Location	Rocky cavities [9]	Cavities in various human-made objects or nest boxes [6]	Rocky nesting cavities in Arctic tundra [7]	Rocky cavities or artificial nest boxes [8]	Rocky cavities on the ground **[1]**	Rocky cavities on the ground **[1]**
Clutch size	5-6 eggs [9]	3-8 eggs [10]	5-7 eggs [11]	5-6 eggs [8,12]	Currently unknown	Currently unknown
# of broods per year	1 [6]	1 [6]	1 [7]	1, but can be 2 if weather conditions are favourable [8]	Currently unknown	Currently unknown
Wintering location	Currently unknown	Currently unknown	Manitoba, Saskatchewan and Alberta [4]	Siberian steppe [5]	N/A	N/A
Breeding season	May-July [13]	May- July [6]	Late May-Aug [7,11]	May-July [8]	May-Sept **[1]**	May-Sept **[1]**

[1]Montgomerie & Lyon, 2020; [2]Macdonald et al., 2012; [3]Fossøy, unpubl. data; [4]McKinnon et al., 2016; [5]Snell et al., 2018; [6]Romero et al., 1998; [7]Guindre-Parker et al., 2013a; [8]Fossøy et al., 2014; [9]Vézina, pers. comm.; [10]Ashley, pers. comm.; [11]Guindre-Parker et al., 2013b; [12]Warner et al., 2019; [13]O'Connor et al., 2021

Table 2.2: Summary statistics for snow bunting (*Plectrophenax nivalis*) samples used for DNA extraction for the breeding population genetics study. These 221 samples were collected from the snow bunting populations during their breeding season (May-September).

Population	Location	Type of Sample	DNA Extraction Method	Specific Region	Year Collected	# of Samples
Alert, NU, Canada (A)	82.30°N, 62.20°W	Dry blood spot on a filter paper	SPRI Beads (Vo & Jedlicka, 2014)	--	2016	13
					2017	38
Svalbard, Norway (S)	78.13°N, 15.38°E	Packed red blood cells (RBC) in ethanol	SPRI Beads (Vo & Jedlicka, 2014)	--	2014	19
					2015	14
Barrow, AK, USA (B)	71.10°N, 156.40°W	Frozen RBC	QIAamp DNA Mini Kit	--	2018	18
		Whole blood and frozen RBC			2019	33
Mitivik Island, NU, Canada (M)	64.01°N, 81.47°W	Dry blood spot on a filter paper	SPRI Beads (Vo & Jedlicka, 2014)	--	2010	31
					2011	19
Aleutian Islands, AK, USA (AI)	51.89°N, 176.64°W	Skin tissue preserved in ethanol	SPRI Beads (Vo & Jedlicka, 2014)	Adak Island	1999	9
	52.89°N, 173.11°W			Attu Island	1999	11
Pribilof Islands, AK, USA (PI)	57.14°N, 170.23°W	Skin tissue preserved in ethanol	SPRI Beads (Vo and Jedlicka 2014)	--	2018	16

RBC: red blood cells, SPRI: solid phase reversible immobilization

Table 2.3: Microsatellite marker pairwise F_{ST} values (below diagonal) and p values for Fisher's exact test of population differentiation (above diagonal) for six snow bunting (*Plectrophenax nivalis*) breeding populations. Bold indicates statistically significant differences after sequential Bonferroni correction at 5% level. See Table 2.2 for description of population codes.

	AI	**PI**	**A**	**B**	**M**	**S**
AI	**--**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
PI	**0.091**	**--**	**<0.0001**	**0.0011**	**<0.0001**	**<0.0001**
A	**0.100**	**0.036**	**--**	**<0.0001**	**<0.0001**	**0.0001**
B	**0.094**	**0.035**	**0.011**	**--**	**<0.0001**	0.0111
M	**0.095**	**0.039**	-0.0001	**0.012**	**--**	**<0.0001**
S	**0.081**	**0.028**	**0.012**	-0.0001	**0.009**	**--**

Table 2.4: SNP loci pairwise F_{ST} values (below diagonal) and p values for Fisher's exact test of population differentiation (above diagonal) for six snow bunting (*Plectrophenax nivalis*) breeding populations. Bold indicates statistically significant differences after sequential Bonferroni correction at 5% level. See Table 2.2 for description of population codes.

	AI	**PI**	**A**	**B**	**M**	**S**
AI	--	0.009	**0.0009**	**<0.0001**	**<0.0001**	**<0.0001**
PI	0.021	--	**<0.0001**	**0.0008**	**<0.0001**	**<0.0001**
A	**0.042**	**0.051**	--	0.7705	0.8786	0.6237
B	**0.035**	**0.039**	0.004	--	0.5645	**0.0008**
M	**0.042**	**0.047**	0.008	0.012	--	0.5531
S	**0.053**	**0.044**	0.013	**0.024**	0.005	--

Table 2.5: Selection effects on a subset of functional SNP loci among six snow bunting (*Plectrophenax nivalis*) breeding populations assayed at the global and pairwise levels. The 11 SNP loci were selected based on having selection status data for all possible pairwise comparisons (See text for more detail). For each SNP locus the associated gene, type of variant, specific SNP, amino acid substitution and gene function category are given. The gene function categories include: Energetics (E), Cellular Housekeeping (H), Lipid Metabolism (LM), Nervous System Development (NS), Reproduction (R), and Stress (S). For each SNP locus, divergent selection (black), stabilizing selection (green), or genetic drift (blue) is shown based on 99% and 99.9% neutral marker confidence intervals for global and pairwise comparisons, respectively. See Table 2.2 for description of population codes.

Primer Name	Gene Description	Type of Variant	Nucleotide Variant	Amino Acid Variant	Category	Global Comparison	Populations Compared														
							AI/PI	AI/A	AI/B	AI/M	AI/S	PI/A	PI/B	PI/M	PI/S	A/B	A/M	A/S	B/M	B/S	M/S
SNP_10	Serine/threonine-protein kinase LATS2	Missense	C/T	Ser/Asn	H																
SNP_13	DNA repair protein complementing XP-C cells	Missense	G/A	Arg/Lys	H																
SNP_100	Corticotropin-releasing factor receptor 1	Upstream	G/A	-	H																
SNP_156	Hexosaminidase D	Downstream	A/G	-	LM																
SNP_41	Activin receptor type-2A	Missense	A/G	Ser/Pro	LM																
SNP_105	Ankyrin repeat and LEM domain-containing protein 2	Downstream	G/A	-	NS																
SNP_56	Activated CDC42 kinase 1	Missense	G/A	Val/Met	NS																
SNP_175	Protocadherin gamma-C5	Synonymous	G/A	Pro/Pro	NS																
SNP_24	BTB/POZ domain-containing protein KCTD17	Missense	T/A	Cys/Ser	R																
SNP_60	Receptor-type tyrosine-protein phosphatase zeta	Missense	A/C	His/Pro	R																
SNP_140	Transcription regulator protein BACH2	Downstream	T/C	-	S																

Figures

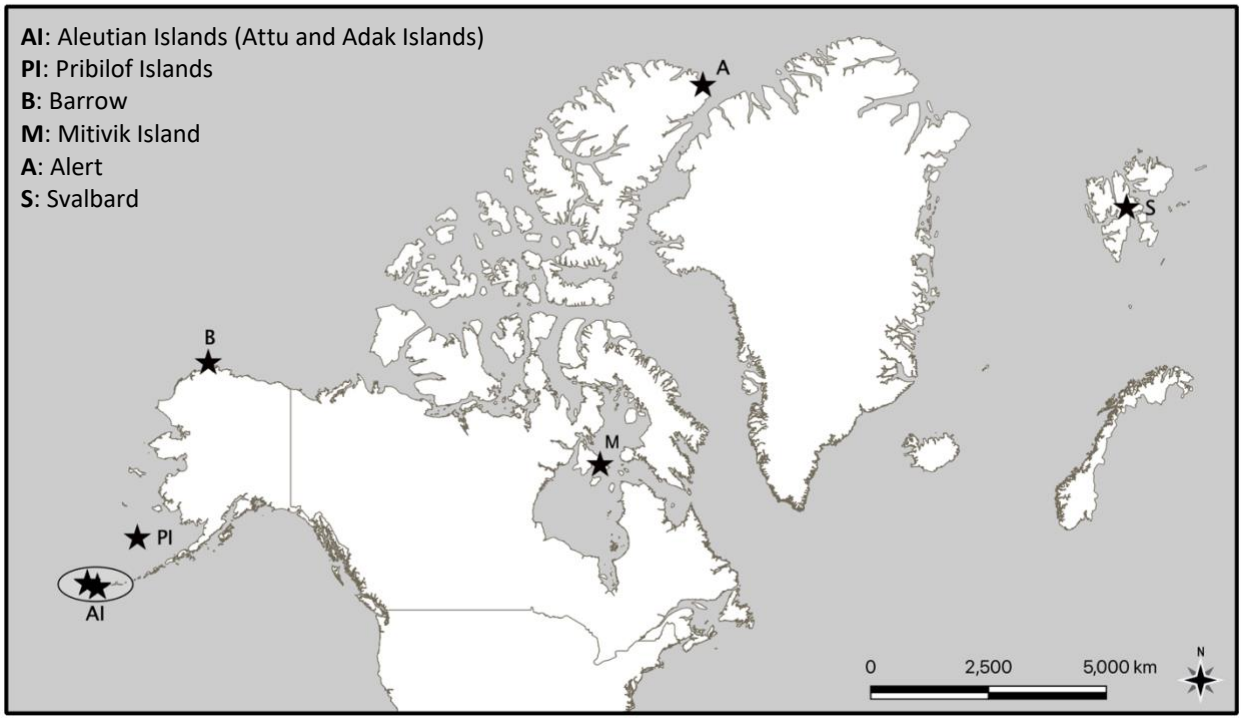


Figure 2.1: Map showing the snow bunting (Plectrophenax nivalis) sampling sites (as indicated by stars) for the breeding population genetics study. Map created using the Free and Open Source QGIS. See Table 2.1 for descriptions of sample locations.

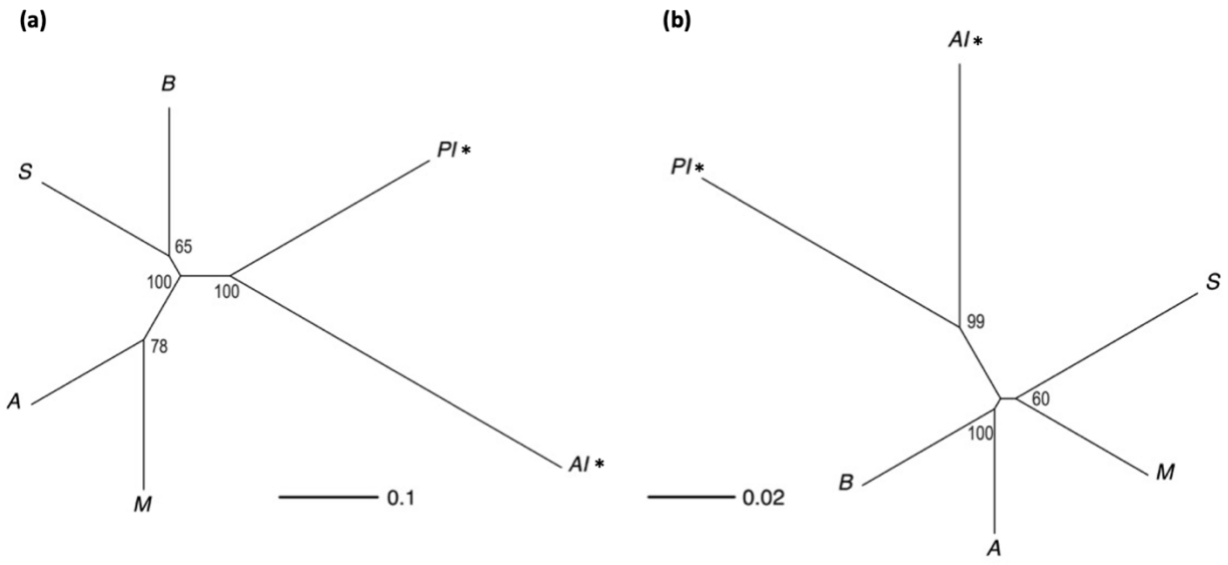


Figure 2.2: Unrooted neighbor-joining cluster analysis diagrams of snow bunting (*Plectrophenax nivalis*) breeding populations based on Cavalli-Sforza and Edwards' (1967) chord distance for microsatellite (Panel a) and SNP (Panel b) markers. The data were bootstrapped over loci with replacement, using 10000 permutations; numbers at branch sites represent the bootstrap support (%) of the branch (support less than 50% is not shown). Asterisks represent non-migratory populations, others are migratory populations. See Table 2.2 for description of population codes.

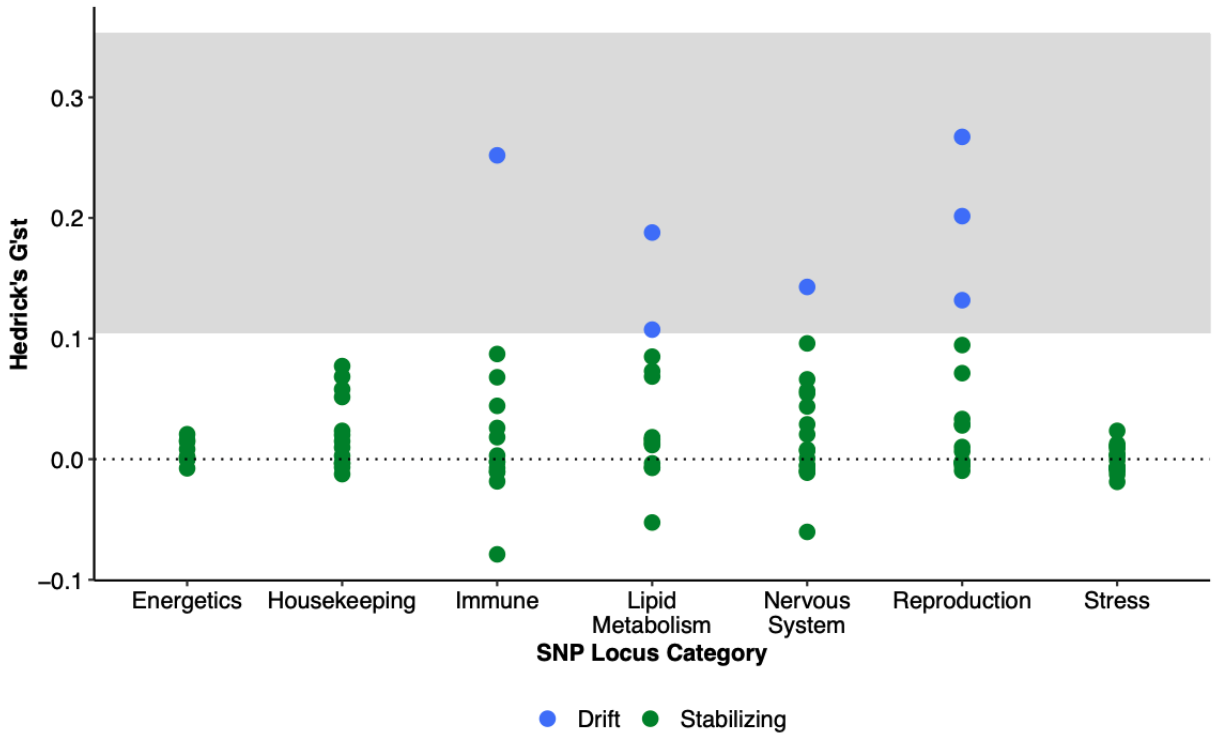


Figure 2.3: Distribution of global Hedrick's G'_{ST} values across the six sampled snow bunting (*Plectrophenax nivalis*) breeding populations for each of the 101 functional SNP loci used in the study. The SNP marker genes were selected from seven broad putative gene function categories. The 99% confidence interval range for neutral divergence (i.e., genetic drift based on microsatellite marker data G'_{ST} values) is shown in grey.

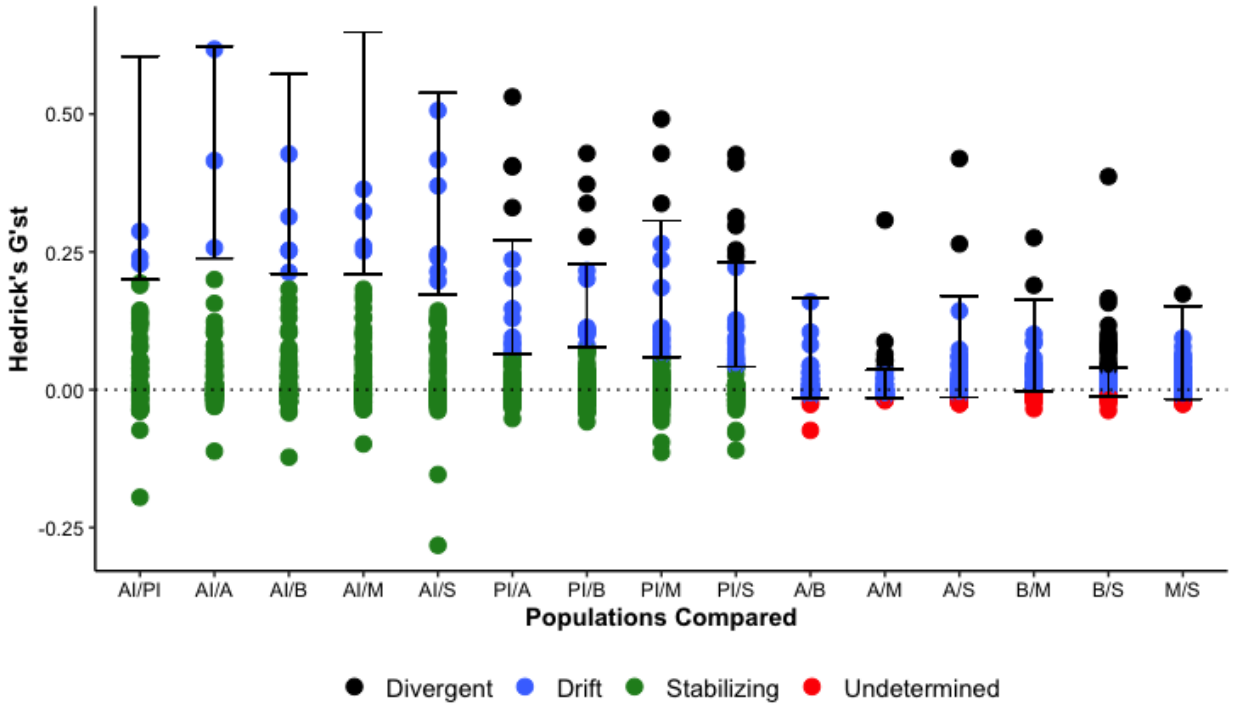


Figure 2.4: Distribution of pairwise Hedrick's G'_{ST} values for the sampled snow bunting (*Plectrophenax nivalis*) breeding populations based on the 101 functional SNP loci. Pairwise comparisons identified as under genetic drift (blue dots) fall within the 99.9% neutral microsatellite marker confidence interval range (shown as error bars for each population comparison). Divergent (black dots) and stabilizing (green dots) selection were determined using the same neutral CI. It was not possible to determine selection status ("Undetermined'; red dots) due to the neutral microsatellite range including zero. See Table 2.2 for description of population codes.

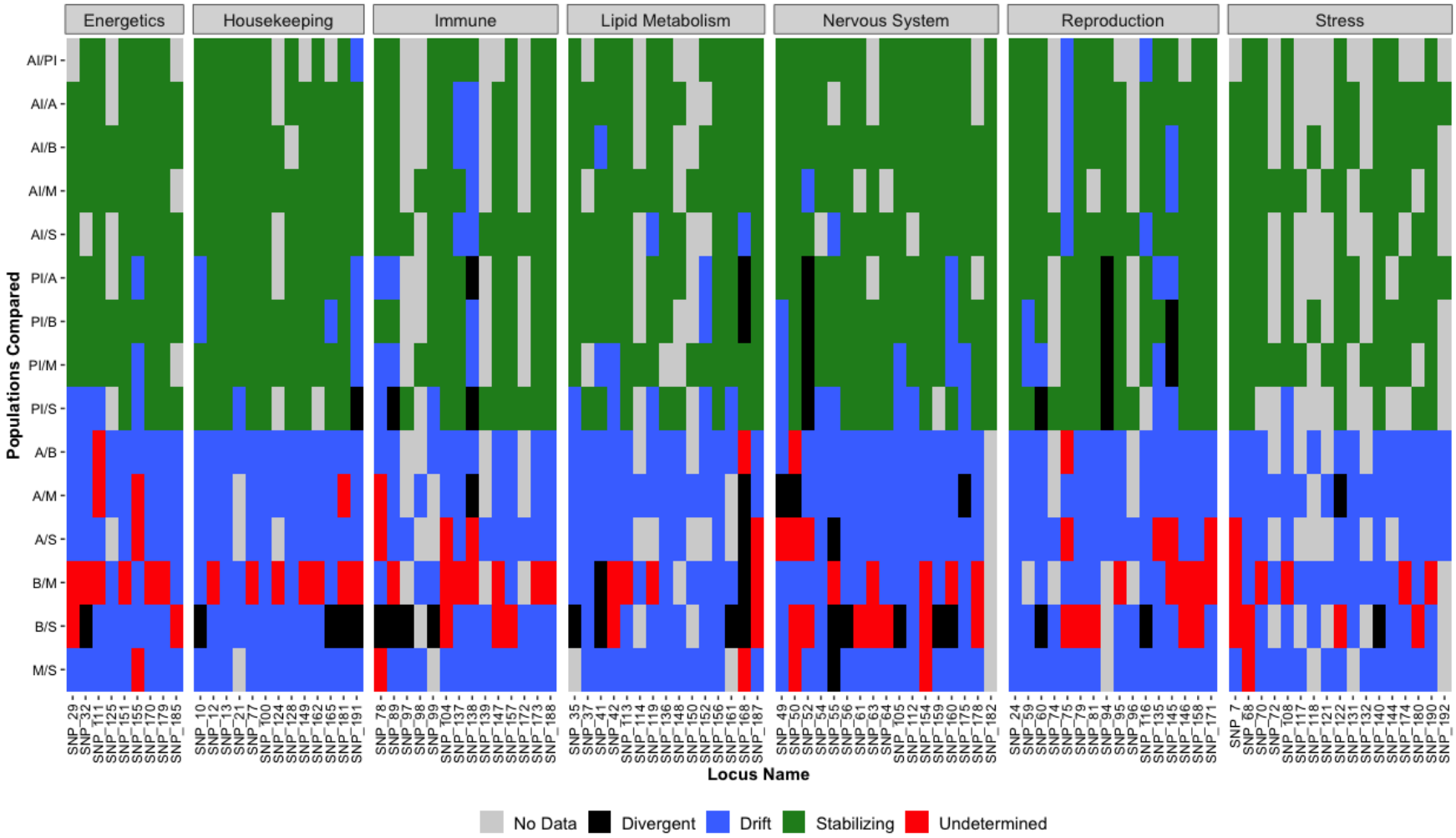


Figure 2.5: Summary map of SNP marker selection status for all pairwise comparisons of the six *Plectrophenax nivalis* breeding populations. All SNPs selected belong to one of the gene function categories as shown at the top of the figure. All selection status results are based on pairwise Hedrick's G'_{ST} value comparisons with the 99.9% neutral marker range. For some pairwise comparisons, we could not estimate Hedrick's G'_{ST} values (grey squares; "No data"), likely due to insufficient sequence reads. The red squares (Undetermined) are pairwise comparisons where the neutral CI range included zero, making stabilizing selection impossible to detect. See Table 2.2 for description of sampled population codes.

CHAPTER 3

ASSESSING THE IMPACT OF MALE QUALITY IN WITHIN- AND EXTRA-PAIR REPRODUCTIVE SUCCESS IN AN ARCTIC BREEDING SONGBIRD

Introduction

Mating systems differ widely across taxa, ranging from monogamy, polygamy (including polygyny, polyandry, and polygynandry), and promiscuity (Clutton-Brock, 1989; Johnson & Burley, 1998; Shuster & Wade, 2019). This diversity reflects mate choice decisions and hence how both sexual and natural selection ultimately influence phenotypic traits in both sexes (Emlen & Oring, 1977; Arnold & Duvall, 1994; Jennions & Petrie, 1997; Bateson & Healy, 2005; Ah-King & Gowaty, 2016). In addition, even within mating systems, variation can still occur (e.g., socially monogamous species which exhibit genetic polygyny; Oliveira et al., 2014; Brouwer & Griffith, 2019; Freeney & Riehl, 2019; Sinervo et al., 2020). Since birds display a variety of mating systems, they are excellent candidate species to examine variation in mating behaviours and their impacts on fitness, and therefore ultimately the evolution of mating systems (Orians, 1969; Wittenberger, 1979; Wink & Dyrcz, 1999). Social monogamy is the most common mating system among passerine birds (Black, 1996; Griffith et al., 2002). It involves the male and female forming a pair bond, where males may defend a breeding territory and feed their incubating female, with both parents then providing care for the young (Lack, 1968; Emlen & Oring, 1977; Kvarnemo, 2018). Traditional approaches to quantifying total male fitness assumed that socially monogamous species also shared true genetic monogamy (i.e., all offspring in a given nest are offspring of the social male of that nest). However, advances in molecular genetic techniques have revealed that this is rarely the

case and that extra-pair paternity (EPP) is in fact a very common occurrence in avian species in general (>500 studies in >300 bird species), with 76% (from 255 species studied to date) occurrence in socially monogamous avian species (Macedo et al., 2008; Biagolini-Jr at al., 2017; Brouwer & Griffith, 2019). Defined as the offspring resulting from promiscuous mating outside of a socially monogamous breeding pair (Westneat et al., 1990), EPP occurs when the social male has offspring outside of the monogamous bond pair, resulting in extra-pair offspring/young (EPO/EPY). Consequently, EPO/EPY are biologically unrelated to the social male of a particular nest and therefore reproductive fitness of socially monogamous males is a combination of offspring sired from both within-pair and extra-pair copulations. This inclusion of the occurrence and degree of EPP is very important in the study of mating systems as it allows for the assessment of both within-pair reproductive success (WPRS) and extra-pair reproductive success (EPRS) contributions to realized fitness, or the sum of all offspring sired by a male in his social nest and through promiscuous mating (i.e., total reproductive fitness - TRS).

The high rates of EPP in songbirds in general (Westneat & Stewart, 2003) combined with the high degree of intra-specific variation in EPP rates (Griffith et al., 2002) offer intriguing questions regarding the underlying mechanisms responsible for this variation within a group of birds that share a similar socially monogamous mating system (Bennet & Owens, 2002; Westneat & Stewart, 2003). However, despite the assumed linkage between variation in male phenotypic quality and variation in both WPRS and EPRS (e.g., meta-studies by Griffith et al., 2002; Cleasby & Nakagawa, 2012), we still know little about the precise reasons behind inter-specific variation in EPP (Griffith et al.,

2002; Crouch & Mason-gamer, 2018; Brouwer & Griffith, 2019). Generalizing these assessments across species has been further complicated by the fact that most data are available for species in the North Temperate Zone, where phenotypic mechanisms in understudied tropical and polar species may play very different roles or have different degrees of impact given differences in environmental constraints (Stutchbury & Morton, 2001; Macedo et al., 2008; Hoset et al., 2013). Quantifying the role(s) of underlying phenotypic mechanisms is an especially intriguing challenge that involves examining what factors contribute to intra-specific variation in EPP and hence how variation in male 'quality' ultimately impacts the relative contributions to intra-specific variation in realized fitness (Griffith et al., 2002). An early review of twenty-three avian studies concluded that intra-specific variation in EPRS could be partially explained by the age, size and condition, dominance, song quality and sexual ornamentation of males (Griffith et al., 2002). Since males receive a direct benefit by engaging in extra-pair copulations (i.e., improved realized fitness without the cost of parental care), we would expect a potential relationship between male phenotypic characteristics and EPRS, especially if female choice is strong (e.g., Griffith et al., 2002; Webster et al., 2007; O'Brien & Russell, 2011). As such, the relative occurrence of EPP can generate strong levels of sexual selection in socially monogamous systems (Griffith et al., 2002; Westneat & Stewart, 2003; Whittingham & Dunn, 2005; Bitton et al., 2007).

While EPP has the potential to increase variance in realized reproductive success by providing opportunities for sexual selection (Richardson & Burke, 2001; Whittingham & Dunn, 2005; Poesel et al., 2011; Schlicht & Kempenaers, 2011), the influence of within-pair paternity (WPP) on sexually selected traits in socially monogamous birds is

relatively unexplored (O'Brien & Russell, 2011). A likely reason behind this is that the majority of sexual selection studies relating male quality traits to reproductive fitness focus only on the EPRS component (Griffith et al., 2002; Andersson & Simmons, 2006), and that studies have generally not discovered any significant and consistent links between intra-specific variation in male quality and WPRS (Kleven et al., 2006; Webster et al., 2008; although see Doucet et al. 2005). Interestingly, there is some evidence suggesting that predictors of EPP gains are entirely different than that of EPP losses for a particular male, suggesting that different phenotypic traits predict variation in EPRS and WPRS (e.g., Lehtonen et al., 2009). These results indicate that females may use a complex set of differential male quality cues when engaging in mixed reproductive behaviour strategies. However, since female pursuit of EPP can be explained by both adaptive (i.e., improving quality of their progeny, infertility insurance, access to resources) and mal-adaptive explanations (i.e., reduced parental care and protection for offspring by cuckholded males, risk of contracting sexually transmitted diseases), it is often difficult to assign general support for hypotheses surrounding the mechanisms driving variation in EPP (Forstmeier et al., 2014). Given the spatiotemporal constraints that mate guarding by social partners may impose, females seeking extra-pair mating opportunities are expected to rely extensively on evaluating extra-pair males based on phenotypic characteristics that can be quickly assessed and act as reliable signals of male quality (Guindre-Parker et al., 2013b), such as plumage quality (Bitton et al., 2007; Balenger et al., 2009; O'Brien & Russell, 2011), song structure and complexity (Gil et al., 2007; Hill et al., 2011), and even body size (Hoset et al., 2014; Wells et al., 2015).

In this study, we examined the link between intra-specific variation in male phenotypic quality and outcomes for WPRS, EPRS and hence realized fitness (i.e., TRS) in an Arctic-breeding population of snow buntings (*Plectrophenax nivalis*). This species is particularly relevant for studying the mechanisms at the heart of variation in male reproductive success for a number of reasons. First, the short Arctic breeding season (i.e., usually only one possible breeding event per year due to a short seasonal breeding period, ephemeral peaks in resource availability and high competition for access to mates; Hoset et al., 2014) generates environmental constraints that may strengthen sexual selection on male phenotypic traits as signals of male quality. Second, male snow buntings have a number of advertisement traits that exhibit significant intra-specific variation, making it easy for females to assess even at a distance (e.g., plumage and song), and which have already been linked to broad measures of breeding decisions and success (Guindre-Parker et al., 2013a; Guindre-Parker et al., 2013b; Guindre-Parker et al., 2013c; Guindre-Parker & Love, 2014; Baldo et al., 2014; Baldo et al., 2015). Finally, preliminary data on sperm quality in snow buntings suggested medium to high rates of EP young in this species (Love & Alchin, unpublished) based on the positive relationship between inter-specific variation in sperm morphology and rates of EPY (Lifjeld et al. 2010). Few studies have taken an integrative approach to examining condition-dependent links between male quality WPRS and EPRS in passerine species in general (e.g., Doucet et al., 2005; Chaine & Lyon, 2008; Hill et al., 2010; O'Brien & Dawson, 2010). Moreover, there have been no studies linking variation in male quality to variation in WPRS in snow buntings, and only one study that has attempted to directly link variation in EPRS to male quality traits,

which revealed a positive correlation between EPP rates and both age and body size of social males (Hoset et al., 2014).

In the current study, we used nine microsatellite markers to determine parentage and estimate the occurrence of EPP, we then use this estimate for the assessment of realized fitness (WPRS and EPRS) of all males in our breeding population. We then explored the relationship between both WPRS and EPRS and a diversity of male phenotypic quality traits. We included a diversity of male traits because females may be assessing males for a range of quality traits simultaneously, and the selected traits are expected to be important drivers of variation in reproductive performance in passerines (Griffith et al., 2002; Guindre-Parker & Love, 2014). More specifically, we examined the relationship between WPRS and EPRS and multiple phenotypic measures such as male advertisement signals (e.g., plumage traits, wing patterns, song traits and measures of territory quality), as well as physical and physiological traits that are likely to be honest indicators of 'quality' (e.g., arrival body mass, levels of circulating immunoglobulins, testosterone, and oxidative stress) (Guindre-Parker et al., 2013a; Guindre-Parker et al., 2013b; Guindre-Parker et al., 2013c; Guindre-Parker & Love, 2014). For males, mixed reproductive behaviour strategy depends on social and ecological factors such as breeding density and synchrony (e.g., Stutchbury & Morton, 1995; Thusius et al., 2001; Hoset et al., 2004; Stewart et al., 2010) in addition to inherent male quality, however the impacts of these two factors on overall fitness are equivocal. While we do not focus on these factors for this specific study, we expected EPRS and WPRS to show positive and negative relationships, respectively, with male quality (Table 3.1) due to expected increases in preference of non-social females for high quality males. As such, we also

expected high quality males to generally increase their EPRS at the possible expense of WPRS (given that males investing in EPRS may lose WPRS to other EPP males). Our study will not only contribute to our current understanding of the relationship male quality and reproductive success, but will give us insight into factors that cause genetic promiscuity in currently understudied socially monogamous Arctic-breeding passerines.

Methods

Study species, system and field procedures

Snow buntings are found in the circumpolar Arctic during the spring and summer (Montgomerie & Lyon, 2020). Males arrive earlier to the breeding grounds than females to gain access to high-quality nesting sites among the rocky cavities in the tundra (Montgomerie & Lyon, 2020). At Mitivik (East Bay) Island (64.01N, 81.47W; located within the Qaqsauqtuuq (East Bay) Migratory Bird Sanctuary, Nunavut, Canada), the breeding and migratory ecology of snow buntings has been studied since 2007 (Baldo et al., 2015; Macdonald et al., 2015; McKinnon et al., 2016). The study population on this island has one of the highest known breeding densities of snow buntings worldwide (~70 pairs/km^2; Love unpubl. data), coincident with the high abundance of granite rocky cavities, which are preferred nesting sites for this species (Montgomerie & Lyon, 2020), as well as an abundance of arthropod prey during the chick-rearing period (Love, unpublished data). Although females are exclusively responsible for incubating eggs, males provide the female food during incubation, and both sexes contribute to provisioning the young (Montgomerie & Lyon, 2020).

In 2010 and 2011, all breeding pairs were captured and given unique metal and colour bands upon their arrival from spring migration (late May to early June; Guindre-

Parker et al., 2013c). In addition to marking all the individuals, breeding territories were mapped, lay dates were assessed, clutch sizes were recorded, and reproductive success was measured (i.e., number of successful hatchlings and fledglings). Blood samples were collected from the brachial vein from all known breeding adults to ensure a complete dataset of all possible male and female parents for parentage analysis (i.e., there were no non-breeding birds, nor any undocumented breeding birds to the best of our knowledge). Blood samples were similarly taken from all chicks (Guindre-Parker et al., 2013c). Blood samples from 2010 and 2011 were preserved on filter paper (approximately 4cm x 1cm) and stored at -20˚C until laboratory analysis. Specifically, the data encompassed 17 adult breeding pairs and 90 chicks from 2010, and 13 adult breeding pairs and 54 chicks from 2011.

Male phenotypic traits

We examined a diversity of male phenotypic traits that were expected to represent different facets of intrinsic (e.g., state) or extrinsic (e.g., physical advertisement signals) male 'quality' metrics (Table 3.1). Our operational definition of 'male quality' is adapted from Guindre-Parker et al. (2013a): "*the ability to maintain homeostasis through changing environments or life-history stages, and the fitness-related consequences of this ability*". Individual male traits represented five broad categories and were chosen based on previous studies in this breeding population and other passerine species suggesting their general importance for predicting variation in broader breeding decisions and success, they include: i) male state at arrival on the breeding grounds (body mass, plasma testosterone, plasma immunoglobulin Y (IgY), and plasma oxidative status); (Guindre-Parker et al., 2013a; Guindre-Parker et al., 2013c; Baldo et al., 2015); ii) song quality

(song structure and complexity); (Baldo et al., 2014; Baldo et al., 2015); iii) wing patterns (spotting, extremity, and alula); (Guindre-Parker et al., 2013a; Guindre-Parker et al., 2013b); iv) plumage quality (breast and mantle plumage); (Guindre-Parker et al., 2013a; Guindre-Parker et al., 2013b); and v) territory quality (territory size and rock cover); (Guindre-Parker et al., 2013a). With regards to variation in male state, we chose to include arrival body mass given its strong positive relationship to body condition (Guindre-Parker et al., 2013b) and the fact it represents the majority of the variation in body condition (i.e., is interchangeable). We included plasma testosterone measured in males between arrival on the breeding grounds and territory establishment given its role in male aggressive interactions and territory defense (Guindre-Parker et al., 2013a). We also examined a general measure of immune system function (IgY) given its role in assessing immune status and that it has predicted offspring fledging success in this species (Guindre-Parker et al., 2013b). Finally, we included blood plasma measures of oxidative status (i.e., oxidative stress and antioxidant capacity) given its role in the production of honest sexual signals (Baldo et al, 2015), territory quality, and offspring provisioning (Guindre-Parker et al., 2013c). We included metrics of song quality given that males in this species use song to advertise to male competitors and possible female mates (Baldo et al., 2014), the strong degree of inter-individual variation in this advertisement trait (Baldo et al. 2014), and its potential links to physiological workload during breeding (Baldo et al. 2015). With regards to wing patterns, we chose to include black spotting on the white wings due to its role in male arrival condition and potential future reproductive success (Guindre-Parker et al., 2013a). We included the relative size (corrected for wing area) of the black primary feathers on wings given its role in

signalling territory quality (Guindre-Parker et al., 2013a). Finally, we examine the relative size of the alula, an achromatic plumage patch on the wings, given its dependence on body condition and diet quality (Guindre-Parker et al., 2013b), and its role in signalling territory quality in this species (Guindre-Parker et al., 2013a). With regards to body plumage quality, we included reflectance measures of the white breast plumage given its link to variation in immune status and potential future reproductive success (Guindre-Parker et al., 2013a). We also included reflectance measures of the darker mantle plumage given its role in signalling territory quality (Guindre-Parker et al., 2013a). With regards to territory quality, we include territory size given its role in territorial behaviours between neighbours (Guindre-Parker et al., 2013a) and rock cover (i.e., cover area of rocks surrounding nests) given its importance in female nest site choice that relates to buffering of offspring from environmental and predation threats (Guindre-Parker et al., 2013a). Detailed descriptions of each specific trait and how each was derived can be found in the respective published papers referenced within each quality category above.

DNA extraction and genotyping

Genomic DNA was extracted from the blood tissue using the commercially available Wizard Kit (Promega Corporation, Madison, WI, USA). We genotyped all offspring and adults at nine microsatellite markers developed for this species (see Chapter 2, Appendix A5) using the detailed protocol described in Chapter 2 of this thesis. Briefly, all DNA samples were amplified at nine microsatellite loci through one round of multiplex PCR for preamplification of DNA (due to of limited quantity of DNA recovered from some individuals) followed by a second round of PCR with individual microsatellite primers.

Each sample was then genotyped on a SeqStudio Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with GeneMapper software v3.5. All samples with genotypes available for 4 or more loci were used for subsequent analyses with CERVUS 3.0.7 (Marshall et al., 1998; Slate et al., 2000; Kalinowski et al., 2007; Kallinowski et al., 2010).

Maternity and paternity assignment

We initially assumed the putative mother was the biological mother for all offspring. We validated this assumption by examining any allele mismatches between putative mothers and their offspring. Although intraspecific brood parasitism (Arnold & Owens, 2002), also known as egg dumping by females into the nests of other females, has never been documented in our study species, to increase our confidence in mother-offspring log likelihood (LOD – see below for more detail) scores and to investigate the assumption of absence of brood parasitism in this species, we also conducted maternity analysis for all offspring. For this, we used the 'Maternity Analysis' option in CERVUS and set the simulation parameters to 95% sampling rate of candidate mothers and 1% genotyping error across all samples. Based on these criteria, we were able to conduct all maternity assignments at a minimum of 95% confidence. We used the candidate fathers assigned to each offspring (see details below) as their known biological fathers and assigned a most-likely mother for each offspring from our pool of candidate mothers. We then compared how well the social mothers and CERVUS-assigned mothers matched based on mother-offspring pair LOD scores.

We performed the paternity analysis for all offspring using 'Paternity Analysis' option in CERVUS. We also used CERVUS to calculate the number of alleles (k),

observed (H_{obs}) and expected (H_{exp}) heterozygosities, polymorphic information content (PIC), probabilities of non-exclusion (NE), test for Hardy Weinberg Equilibrium, and null allele frequency at each locus (Table 3.2). Briefly, paternity assignment involved using microsatellite marker genotypes to assign a true biological father to each offspring out of the pool of candidate fathers in our study population, given that the social mother was also the biological mother. We defined 'candidate fathers' as all known breeding males in our study population within a specific year. Simply, paternity analysis involves a process of exclusion where the genotypes of candidate fathers are compared against offspring genotypes at all loci (taking the maternal genotypes and any genotyping errors (1% as entered into paternity simulation) into account), and candidate males are excluded as potential fathers if mismatch occurs at one or more loci. While complete exclusion is a powerful method to assign paternity when few candidate parents and highly polymorphic markers are available, it is not always feasible (Jones et al., 2010). To resolve this issue, CERVUS uses a categorical allocation approach to assign paternity, since it adds additional confidence to the process of exclusion by using the Log-odds (the natural log of the likelihood ratio; 'LOD' here-onwards) scores. A positive LOD score indicates that the candidate father is much more likely to be the true biological father, whereas a negative LOD score indicates that the candidate father is unlikely to be a true biological father from a given pool of candidates (due to allele mismatches at one or more loci).

Since the sampling of the candidate parents in the study area was exhaustive during both study years, we therefore used a sampling rate of 100% and 99% in CERVUS for the social males and females, respectively. Our choice of slightly lower confidence (99%) in the sampling of breeding females is to account for the fact that a

non-sampled female may have arrived at the island later on in the season and mated with any of the breeding males; however, we feel this is not a likely possibility, but using a sampling rate of 99% allows for that possibility. Additionally, the paternity assignments for individual offspring were carried out at 95% (strict) and 80% (relaxed) confidence levels (Jones et al., 2010). Using these parameter settings in CERVUS, we ran a simulation of 10,000 randomly generated offspring based on parental genotypes and produced the distribution of LOD scores and confidence levels for assignment of most-likely candidate fathers. We used the trio-LOD scores when assigning parentage to all chicks in our study area. The trio-LOD score is calculated for the father-offspring relationship given our confidence in mother-offspring relationships. Considering the results from the paternity simulation, all paternity assignments achieved a minimum of 94.7% and 96.2% confidence levels in 2010 and 2011, respectively.

Reproductive success: three matrices

We used the paternity assignment results to calculate within-pair and extra-pair reproductive success for each male. The "within-pair reproductive success" (WPRS) for a given male was the number of offspring he sired in his social nest, whereas the "extra-pair reproductive success" (EPRS) for a given male was the total number of offspring he sired in the nests of other males within that breeding season. Additionally, we calculated each male's annual 'total reproductive success' (TRS) as the sum of WPRS and EPRS for that breeding year. Finally, we also considered 'EPRS Allocation' (i.e., the number of nests with at least 1 EPY) as a second reproductive matrix for estimation of extra-pair reproductive success to explore whether the males spread their EPY reproductive effort across multiple nests.

Statistical analyses

To investigate whether variation in male quality predicted variation in reproductive success in snow buntings, we ran three Generalized Linear Models (GLM) using male quality metrics to predict variation in our three measures of reproductive success: i) WPRS, ii) EPRS, and iii) EPRS Allocation. Although we initially (and ambitiously) hoped to include all 20 male quality phenotypic traits within each reproductive success model, not surprisingly we ran into problems with over-fitting and over-dispersion of our GLM models due to small sample size (only 30 males across two sampling years) and too many independent variables. We were then required to make one of two choices to continue: eliminate individual male traits, or attempt to collapse male traits down into functional groups. Since the primary goal of our analyses was to broadly assess male quality (i.e., male phenotypic quality; Guindre-Parker et al., 2013a) in relation to reproductive success, without inadvertently excluding any male quality traits, we decided on the latter strategy. We therefore used a Principle Component Analysis (PCA) approach to reduce the 20 individual male traits to five functional groups using a two-stage PCA (see details below). This type of two-stage approach has been successfully used previously (e.g., Dender et al. 2018). While this technique has the drawback of reducing the ability to directly examine how individual male traits predict reproductive success, our overall goal was to examine the relationship between broader male phenotypic variation and reproductive success, and this approach allowed us to examine multiple individual male quality traits without suffering from serious multiple comparison testing errors.

All male quality trait response variables (Appendix B1) were tested to confirm they met assumptions of normality and homogeneity. Only "Male arrival testosterone" required data transformation (Box-Cox; Box & Cox, 1964). All male quality traits were then standardized using Z-score transformation to allow for statistical comparison of different male quality traits. Next, we grouped the 20 male quality traits *a priori* into one of five functional categories - male state, song quality, wing pattern, body plumage and territory quality - as shown in Appendix B1 and based on previous work in this species (see trait details above). We then used a PCA approach to collapse multiple traits within each of the five categories down to one or two principle component axes using varimax factor rotation (all selected PC factors had eigenvalue scores of >1; Appendix B1). Each extracted component explained a minimum of 30% of variance in a given male quality category (see Appendix B1 & B2) for specific trait variances and PC interpretations). We subsequently conducted a second application of PCA to further collapse our male quality groups. From the resulting components, we extracted PC1-3 from this second stage of PCA for subsequent GLMs. The PC4 term was solely represented by one factor (Song Complexity) with a heavy factor loading (0.91). We therefore included the original Song Complexity component extracted from first stage of PCA along with PC1-3 in our final GLMs. This approach therefore produced four final components (Appendix B3 & B4) with eigenvalue scores of >1 which were included to represent male phenotypic quality in the subsequent reproductive success GLMs.

We used GLMs with a Poisson distribution and a log-link function (given our data are count data) for our three primary models examining WPRS, EPRS, and EPRS Allocation as our dependent variables (referred to as WPRS, EPRS, and EPRS Allocation

models, respectively, hereon in). More specifically, our aim was to model these three dependent variables as a function of the four independent male quality variables (i.e., PC1-3 from second stage of PCA, Song Complexity PC from first stage of PCA) generated from the two-stage PCA approach. All models included "male arrival date" as a fixed effect covariate to account for differential male arrival dates causing downstream impacts on pairing phenology and hence laying phenology and reproductive success. For example, if an early-arriving male pairs with a female and initiates his clutch earlier in the breeding season, he may be free from constraints of mate guarding, and may have more chances to get EPP once his social partner has begun incubation (van Dongen & Mulder, 2009). We also included the "number of male neighbors", or the total number of males that a focal male's territory was associated with, as a covariate in both component models given that the density of neighboring males could affect both a focal male's own WPRS (via EPP losses), as well as his ability to gain EPP as additional offspring via EPRS. While we additionally attempted to include this trait within the original "Territory Quality" PCA, the term remained important on its own with a strong factor loading and did not group with the other components of the territory PCA. Finally, to control for variation in male breeding investment, we included "brood size" (e.g., the total number of chicks within a focal male's nest) for the WPRS model, and "WPRS" for the EPRS and EPRS Allocation models. All variables remained in full models regardless of final statistical significance. From the 30 males across the two sampling years, we excluded a total of 6 individuals: three buntings present in both years, given that these males were present in both breeding seasons. We the included the individual year value with most male quality data out of the repeats (males L, U and M form 2010 discarded, as they were

identical to males #A, #H and #B from 2011). Additionally, three other individual males had to be excluded (2010: B & T; 2011: G) due to incomplete male quality datasets. The remaining 24 males were included in all three GLMs. All analyses were completed using JMP version 14 (SAS Institute Inc., Cray, NC, USA) and all results were evaluated for significance at α=0.05 except where indicated.

Results

Allele frequency and polymorphism

The microsatellite marker panel exhibited high polymorphism and confidence for paternity analyses (Table 3.2). The average number of alleles per locus was 12.56 and 11.67, in 2010 and 2011, respectively. Levels of H_{Obs} were high (average values in 2010 and 2011 were 0.64 and 0.70, respectively) and somewhat similar in both years. The PIC was also high in all loci across the two years (Table 3.2). These results were expected for this microsatellite marker panel, which have demonstrated high heterozygosity and polymorphism in snow buntings from six breeding populations (see Chapter 2, Appendix A5). Using this panel of microsatellites, we successfully genotyped all parents (2010: 17 males and 16 females, 2011: 13 males and 13 females) from both years, with the exception of one female from 2010 as we were unable to obtain her blood sample. We discarded all six chicks from her brood from 2010. Four chicks (all from 2011) were removed from further analyses due to lack of genotypes at four or more microsatellite loci. Overall, we used 84 (93.3%) and 50 (92.6%) offspring from 2010 and 2011, respectively, for paternity and maternity analyses since these offspring were scored at 4 or more microsatellite loci.

Analyses of maternity

In general, all females matched well with the offspring in their nest at most loci. For example, analyses revealed a 86.4% (523 of 605 total comparisons) match between offspring and their social mothers in 2010. This proportion was even higher with 97.5% (390 of 400 total comparisons) match in 2011. Of the mother- offspring mismatches, most (2010: 76.83% (63 mismatches) and 2011: 50% (5 mismatches) occurred at loci with null allele frequency of greater than 0.10. Furthermore 19.0% (12 mismatches) and 40.0% (2 mismatches) of these were consistent with the presence of a null allele for 2010 and 2011, respectively. The levels of mother-offspring mismatch were markedly higher in the 2010 breeding season, therefore triggering further maternity analysis using CERVUS after assigning paternity to each offspring. Of 25 offspring (out of 87) that had mother-offspring pair LOD scores of less than -1 (ranging between -1.06 and -8.92) from paternity analyses, 15 chicks were assigned to their social mother (Appendix B5). The remaining 10 chicks were assigned to a different female from the population. Despite this outcome, we continued our paternity analysis with the assumption that the social mothers were the true mothers for all chicks. This assumption does not strongly affect the outcomes of the paternity assignment, plus, given the primary focus of our study was to explore correlative relationships between various male quality traits and different reproductive output matrices, the possibility of a low rate of egg dumping is not critical.

EPP, paternity assignment and reproductive success

All offspring included in the paternity analyses were assigned paternity with approximately 94.7% confidence, resulting in a nearly 100% success rate of paternity assignment at both strict and relaxed confidence levels. Across both study years, many

offspring did not genetically match their social fathers, confirming a very high overall rate of EPP (Table 3.3). Overall, three-fourths of nests from 2010 and approximately half of nests from 2011 contained at least one EPY, with ~36% and 42% EPYs observed across 2010 and 2011, respectively (Table 3.3). The overall rate of WPP was higher across both years. Following paternity assignments, we calculated the total WPRS and EPRS for each male from 2010 and 2011 (Appendix B6). Across both study years, WPRS ranged from 0-6 chicks and EPRS ranged from 0-5 chicks, with TRS ranging from 0-11 chicks (Figure 3.1, Appendix B6). In addition, EPRS allocation also varied in males that were able to secure EPP, ranging from siring all EPYs from a single female (i.e. in one nest) to siring chicks across multiple females (i.e. across 2-4 nests) (Figure 3.1, Appendix B6).

Predictors of WPRS, EPRS and EPRS Allocation

The overall WPRS and EPRS Allocation models were not significant (p=0.230, p=0.156, respectively) (Table 3.4). As such, no strong male phenotypic traits emerged as significant predictors of variation in WPRS and EPRS allocation. The EPRS model was significant (p=0.008), with WPRS (p=0.010) and the Combined PC2 (p=0.001) as positive and negative predictors of EPRS variation, respectively (Table 3.3). Combined PC2 was composed of approximately equal loadings of 'Arrival Body Mass and Testosterone' (-0.77) and 'Breast Plumage' (0.83) PCs from first stage of PCA (Figure 3.2, Appendix B1, Appendix B2). Further deconstruction of these two significant PCs revealed that arrival body mass, arrival testosterone and breast UV chroma were all positive predictors of male EPRS, whereas breast brightness and breast saturation were negative predictors of male EPRS (Figure 3.2). Finally, males with higher WPRS also

tended to have higher EPRS ($F_{5, 17} = 3.25$, p=0.03, Figure 3.1) although the prediction was weak ($R^2_{adj} = 0.025$).

Discussion

Despite the availability of powerful genetic tools for assigning paternity within avian species over decades, little is known about drivers of inter- and especially intra-specific variation in the occurrence of extra-pair paternity. Even less is known about whether male quality differentially predicts variation in within- versus extra-pair reproductive success. In this study, we first quantified extra-pair paternity (EPP) rates and then assessed how variation in a diversity of male quality traits predicted intra-specific variation in within- and extra-pair breeding success in an Arctic breeding population of snow buntings (*Plectrophenax nivalis*). We found high levels of EPP; with 66% of broods containing at least one extra-pair young (EPY), with at least 38% of offspring being genetically unrelated to the social father. We predicted that males scoring higher in quality traits would have lower within-pair reproductive success (WPRS), but subsequently higher extra-pair reproductive success (EPRS) through EPP gains, thus resulting in higher total reproductive success. We assessed the relationship between groups of male quality traits (i.e. principle components; PCs) and WPRS, EPRS and EPRS allocation. While no male quality traits predicted a male's WPRS, we found that males with higher arrival body mass, testosterone and breast UV chroma combined with lower breast brightness and saturation had higher EPRS. Despite this, none of the same quality traits significantly predicted whether the EPP for a given male was concentrated within a given nest or spread out across multiple nests. Here we discuss the occurrence of high rates of EPP in this population and species in general and discuss the significance of

the relationships between male quality and EPRS, and the lack of significant relationships to variation in WPRS.

Rates of extra-pair paternity in snow buntings

Although a common occurrence, EPP rates are generally considered moderate across many socially monogamous avian species, with EPP frequencies averaging at 19% of total offspring being EPY and 33% of broods having at least one EPY (Brouwer & Griffith, 2019). Contrary to the average levels, snow buntings in our focal breeding population showed high levels of EPP occurrences (38% of total offspring being EPY and 66% of broods having at least one EPY). While there are no EPP rates available for snow bunting congeners, our results are somewhat consistent with EPP rates of closely related passerine species, where confamilial species (12 species from Emberizidae family for which EPP data are available) show an average of 50% broods containing EPYs (Bonier et al., 2014). Nonetheless, the EPP frequency reported in our study is much higher than the average frequency reported for this species in an earlier study (11% of total offspring being EPY and 21% of broods having at least one EPY) of a breeding population in Svalbard, Norway (Hoset et al., 2014). A possible explanation for our observed levels of EPP comprise two non-mutually exclusive drivers; high breeding density and a synchronized breeding season. While both of these factors have been important for explaining intra-specific variation in EPP rates within and among multiple conspecific populations of many birds, the evidence has been equivocal (Griffith et al., 2002; Brouwer & Griffith, 2019). For example, breeding density was positively correlated with EPP rates in red-winged blackbirds (*Agelaius phoniceus*) (Gibbs et al. 1990), European pied flycatchers (*Ficedula hypileuca*) (Lifjeld et al. 1991) and yellow

warblers (*Dendroica petechia*) (Yezerinac et al., 1999), but negatively correlated with EPP rates in the great reed warbler (*Acrocephalus arundinaceus*) (Hasselquist et al., 1995; Leisler et al., 2000). There has been only one meta-analysis to the best of our knowledge (comprising 11 passerine species) indicating a positive relationship between breeding density and EPP rates across different populations within a single species (Møller & Ninni, 1998). Although it is not possible to test breeding density as the driver of snow bunting EPP rates given it has been estimated in only two populations, we do know that the breeding density at our Mitivik Island study population is extremely high (~70 breeding pairs/km^2, Love unpubl. data) compared to other snow bunting breeding populations around the world (≤3 breeding pairs/km^2; Montgomerie & Lyon, 2011). Such a high breeding density may be one of the contributing factors to the high occurrence of EPP as it could both facilitate interactions between individuals, as well as make it feasible for a male to visit females in other nearby territories without losing significant paternity within his own nest. Similarly, highly synchronized breeding at Mitivik Island may create opportunities for simultaneous comparison of males by females (Westneat et al., 1990), thereby potentially facilitating EPP in this population (Love, personal communication). However, a population with highly synchronized breeding would also generate trade-offs for males between the benefits of seeking EPP, and the benefits of maintaining high WPP through mate guarding of his social mate. Regardless of the mechanism, high levels of EPP at our study population allowed us to explore intriguing questions regarding the male phenotypic mechanisms that might drive this reproductive flexibility in males.

Male quality predictors of within-pair breeding success

While identification of EPP (i.e., whether offspring in a given nest all share the same genetic father) is a relatively simple procedure, comparatively few studies have assessed realized fitness of males (e.g., Whittingham & Dunn, 2005; O'Brien & Dawson, 2010, Lebigre et al., 2012) since it is not possible to determine sires for all EPY in cases where not all individuals in the study populations are sampled for genetic analyses (Griffith et al., 2002; Brouwer & Griffith, 2019). In the studies that do successfully assess the total reproductive success of males, WPRS (rather than EPRS) is often the dominant factor responsible for a majority of variance in TRS. We found the same general outcome, in addition, males with higher WPRS also had higher EPRS. This finding is intriguing because it suggests that the same male quality mechanisms should be driving both sources of male reproductive success, yet our WPRS and EPRS models did not show the same predictors (in fact WPRS models did not show any significant predictors at all, partially due to our small sample size).

We found that despite examining a diversity of male quality metrics we did not detect any significant relationships between male quality and WPRS. Unfortunately, there are very few studies relating WPRS to male phenotypes/quality to guide discussion since most studies have instead focused on predictors of EPRS as drivers of the evolution of male traits. Of the existing studies, results are equivocal. For example, while a large meta-analysis by Cleasby & Nakagawa (2012) found that older males generally had higher EPRS, they did not find any relationship between age and WPRS in 61 passerine studies. Nonetheless, Doucet et al. (2005) did show that various measures of achromatic plumage predicted WPRS in male black-capped chickadees (*Poecile atricapillus*). The

lack of male quality predictors for variation in WPRS in our study might partially be explained by high breeding density leading to high levels of EPP (discussed above), hence diluting the link between male quality traits and WPRS. This hypothesis can be tested empirically in a more dispersed breeding population of snow buntings where potential male quality predictors of WPRS could emerge.

Male quality predictors of extra-pair breeding success

We found two quality groups (i.e. PCs) as significant drivers of male EPRS variation: arrival body mass and testosterone, and breast plumage. Snow bunting's EPRS was contingent upon his body mass and testosterone levels at the time to arrival on the breeding grounds, as well as the reflectance measurements of his white breast plumage. Body size (i.e., larger body mass) in passerines has been shown to be associated with ability to survive and successfully reproduce. Indeed, larger males often have both higher WPRS (i.e., they lose less paternity within their social nest) and EPRS (Hutchinson & Griffith, 2008; Lehtonen et al., 2009). Although we did not find a relationship between body mass and male's WPRS, we did find that larger/heavier males received higher EPRS. This is consistent with a previous EPP study in the same species that showed larger males investing in EPP pursuit at the expense of losing paternity within their social nests (Hoset et al., 2014), resulting in higher EPRS. Additionally, residual body mass has been considered a positive measure of body condition in snow buntings at Mitivik Island (Guindre-Parker et al., 2013b), and the same study has shown an achromatic plumage signal (alula) as a significant predictor of male condition, and ultimately, the reproductive success (measured by number of fledglings). Although the reproductive success was not portioned into WPRS and EPRS by Guindre-Parker et al. (2013b), their results suggest

that high quality males helped in improving the number of fledglings that leave the nest. This is consistent with our findings as it indicates that females may be preferring larger and heavier males as extra-pair mates to also increase the success of their future offspring. Since we did not investigate WPP losses of EP males, it is unclear whether increased EPRS is in addition to, or at the expense of, the WPP. Similar to body mass, arrival testosterone levels were also positively related male EPRS. High circulating levels of testosterone early in the breeding season may help males establish and defend their territories (Garamszegi et al., 2005), allowing males to initiate an early clutch with his social mate, leaving more time and energy for allocation of EPP later on in the season. This idea is consistent with the findings from experimental studies showing increased polygyny (reviewed in Wingfield, 1984; and Beletsky et al, 1995;) and EPP (Raouf et al, 1997) with supplemental exogenous testosterone. The relationship between EPP gains and increased testosterone may not be direct as testosterone levels in males have been shown to enhance male reproductive displays such as production of song or sexual ornaments (Owens & Short, 1995; Ball et al., 2002; Roberts et al., 2004), which may be driven by female choice, leading to runaway selection on male testosterone levels. Our findings are consistent with this study in the sense that the connection between testosterone levels and EPRS may not be a direct one. In snow buntings breeding at Mitivik Island, arrival testosterone has shown to play a role in intra-sexual aggression and territoriality, allowing males to obtain and defend smaller, but higher quality territories that must be defended from multiple male neighbours (Guindre-Parker et al., 2013a), generating an oxidative cost (Guindre-Parker et al., 2013c; Baldo et al., 2015). Additionally, a positive relationship between testosterone levels and breast UV chroma

(i.e., signature of mounting a higher immune response) has been shown through correlational analyses (Guindre-Parker et al., 2013a). Combining those two findings, we suggest that males with higher testosterone levels likely suffer higher oxidative stress and active immune responses, which together indicate his ability to handle a high physiological workload (as suggested by Baldo et al. 2015). Perhaps this attribute plays a key role in female choice for extra-pair males, resulting in an increased EPRS.

We also found that the reflectance measurements (UV chroma levels) of a male's white breast plumage (i.e., brighter breast feathers) predicted greater gains in EPRS. White plumage signals have been shown to act as an ornament in snow buntings (Guindre-Parker et al., 2013a) and other passerines (Griggio et al., 2011; Zanollo et al., 2012; Badás et al., 2018) as it has the potential to act as an honest, condition dependent signal, especially in combination with other (i.e., black and grey) achromatic plumage patches (McGlothlin et al., 2007; Galdbach et al., 2011; Guindre-Parker & Love, 2014). However, the majority of studies to date have focused on the size of the achromatic plumage patch (e.g., Senar, 1999; Thusius et al., 2001) rather than its reflectance properties (Siitari & Huhta, 2002; Doucet et al., 2005) when investigating its relation to metrics of reproductive fitness. One study investigating the relationship between male realized fitness and achromatic plumage patch in black-capped chickadees showed that whiter and brighter plumage was associated with higher WPRS (Doucet et al., 2005). However, there are no published studies currently relating reflectance of the white plumage patch to EPRS in species with achromatic plumage. Our results suggest males with breast plumage that is lower in brightness and saturation may be more successful in securing EPP; perhaps because they are better able to intrude or sneak on the territories of

other males. Alternatively, these traits may signal good genes to a female for fitness-related traits that have not been measured yet. If this is the case, female choice for lower brightness and saturation could be due to her longer-term fitness gains (i.e., offspring survival or future parental effort the offspring; e.g., Gerlach et al., 2012), which we are currently unable to test for, as our focus is on short-term fitness metrics (i.e., fledging success). Our results on UV breast chroma and its positive relationship with EPRS are in initial disagreement with a previous study on the same individuals showing lower breast UV chroma as a key predictor of increased future reproductive performance (Guindre-Parker et al., 2013a). However, it should be noted that the Guindre-Parker et al. (2013a) study could only relate breast UV chroma to the total number of chicks fledged within a male's social nest (i.e., those chicks may have been any proportion of WP and EP reproductive success, but due to a lack of parentage information were all considered to be his own). While initially contradictory, these anomalous results may instead provide key insight into the different signaling messages that breast brightness provides to social versus extra-pair females. Low breast brightness may indicate a male's parental care abilities for a social female, whereas higher scores of this signal may indicate some additional aspect of inherent quality to an extra-pair female. For example, since higher breast UV chroma levels have been shown to be related to increased immune response in this species through increased IgY levels (Guindre-Parker et al., 2013a), females might be choosing EP males that can mount stronger innate and adaptive immune response rather than their potential for future reproductive performance. Overall, our findings are consistent with the idea that female snow buntings likely use multiple signals simultaneously to assess male quality (Guindre-Parker et al., 2013a). Moreover, our

results combined with those of previous studies in this and other species suggest that female snow buntings are may be differentially using multiple measures of male quality when assessing a particular male as a WP or an EP mate.

Conclusions and future directions

There has been little support for the idea that only a few males from a population sire most of the EPOs (Whittingham & Dunn, 2016), which would result in very strong opportunity for selection. Instead, we found that a large proportion of males in our breeding population of snow buntings sired EPOs (e.g., 75% and 54% from 2010 and 2011, respectively). However, WPRS emerged as a significant predictor of male's overall EPRS, suggesting males that excelled in maintaining paternity in their own social nests were also more likely to succeed in improving their realized fitness through EPP gains. Siring additional EPO for male snow buntings is an advantageous strategy especially if it does not involve any substantial loss of paternity within the male's social nest. Overall, our results suggest that this type of mixed breeding strategy may play an important part in the evolutionary role of male quality traits via links with EPRS in this population, hence possibly enhancing the opportunity for sexual selection. Despite strong relationships between some male quality traits and EPRS, we still observed significant inter-individual variation in EPP gains within our breeding population. More specifically, individuals that gained EPO were not necessarily equal when it came to EPP allocation as the extra-pair reproductive effort was either concentrated to one nest or spread across multiple nests. Indeed, while the vast majority of males in our population gained WPP and some EPP, two males in each of the two study years obtained all of the reproductive success by EPO

alone. Currently however, this inter-individual variation in EPP strategy could not be explained by any of the male quality traits we measured.

In conclusion, this study furthers our understanding of mating strategies of one of the earliest-arriving migratory species of the breeding season. Generally, there is still very little known about the underlying mechanisms contributing to variation in realized fitness across males in passerines, and even less within breeding systems of Arctic-migratory birds. To build upon our findings, future work should address the interactions between the social male, social female and the EP male, in light of changing social and ecological factors due to rapid climate change to enhance our understanding on the evolution of mating behaviours of Arctic-migratory avian species.

References

Ah-King, M., & Gowaty, P. A. (2016). A conceptual review of mate choice: stochastic demography, within-sex phenotypic plasticity, and individual flexibility. *Ecology and Evolution, 6*(14), 4607-4642.

Andersson, M., & Simmons, L. W. (2006). Sexual selection and mate choice. *Trends in Ecology & Evolution*, *21*(6), 296-302.

Arnold, K. E., & Owens, I. P. (2002). Extra-pair paternity and egg dumping in birds: life history, parental care and the risk of retaliation. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1497), 1263-1269.

Arnold, S. J., & Duvall, D. (1994). Animal mating systems: a synthesis based on selection theory. *The American Naturalist*, *143*(2), 317-348.

Badás, E. P., Martínez, J., Rivero-de Aguilar, J., Ponce, C., Stevens, M., & Merino, S. (2018). Colour change in a structural ornament is related to individual quality, parasites and mating patterns in the blue tit. *The Science of Nature*, *105*(1), 1-12.

Baldo, S. (2015). Song as an honest indicator of oxidative damage and anti-oxidant capacity: Exploring the relationships between signal quality, oxidative status, and reproduction in the Snow Bunting (*Plectrophenax nivalis*).

Baldo, S., Mennill, D. J., Guindre-Parker, S., Gilchrist, H. G., & Love, O. P. (2014). Snow buntings sing individually distinctive songs and show inter-annual variation in song structure. *The Wilson Journal of Ornithology*, *126*(2), 333-338.

Baldo, S., Mennill, D. J., Guindre-Parker, S., Gilchrist, H. G., & Love, O. P. (2015). The oxidative cost of acoustic signals: examining steroid versus aerobic activity hypotheses in a wild bird. *Ethology*, *121*(11), 1081-1090.

Balenger, S. L., Johnson, L. S., & Masters, B. S. (2009). Sexual selection in a socially monogamous bird: male color predicts paternity success in the mountain bluebird, *Sialia currucoides*. *Behavioral Ecology and Sociobiology*, *63*(3), 403-411.

Ball, G. F., Riters, L. V., & Balthazart, J. (2002). Neuroendocrinology of song behavior and avian brain plasticity: multiple sites of action of sex steroid hormones. *Frontiers in Neuroendocrinology*, *23*(2), 137-178.

Bateson, M., & Healy, S. D. (2005). Comparative evaluation and its implications for mate choice. *Trends in Ecology & Evolution*, *20*(12), 659-664.

Beletsky, L. D., Gori, D. F., Freeman, S., & Wingfield, J. C. (1995). Testosterone and polygyny in birds. In *Current Ornithology* (pp. 1-41). Springer, Boston, MA.

Bennett, P. M., & Owens, I. P. (2002). *Evolutionary ecology of birds: life histories, mating systems and extinction*. Oxford University Press, Oxford, United Kingdom.

Bensch, S., Price, T., & Kohn, J. (1997). Isolation and characterization of microsatellite loci in a *Phylloscopus* warbler. *Molecular Ecology*, *6*(1), 91-92.

Biagolini Jr, C., Westneat, D. F., & Francisco, M. R. (2017). Does habitat structural complexity influence the frequency of extra-pair paternity in birds?. *Behavioral Ecology and Sociobiology, 71*, 1-8.

Bitton, P. P., O'Brien, E. L., & Dawson, R. D. (2007). Plumage brightness and age predict extrapair fertilization success of male tree swallows, *Tachycineta bicolor*. *Animal Behaviour*, *74*(6), 1777-1784.

Black, J. M. (Ed.). (1996). *Partnerships in birds: the study of monogamy: the study of monogamy*. Oxford University Press, UK.

Bonier, F., Eikenaar, C., Martin, P. R., & Moore, I. T. (2014). Extrapair paternity rates vary with latitude and elevation in Emberizid sparrows. *The American Naturalist*, *183*(1), 54-61.

Box, G. E., & Cox, D. R. (1964). An analysis of transformations. *Journal of the Royal Statistical Society: Series B (Methodological)*, 26(2), 211-243.

Brouwer, L., & Griffith, S. C. (2019). Extra-pair paternity in birds. *Molecular Ecology*, *28*(22), 4864-4882.

Burke, T., & Bruford, M. W. (1987). DNA fingerprinting in birds. *Nature*, *327*(6118), 149-152.

Chaine, A. S., & Lyon, B. E. (2008). Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the lark bunting. *Science*, *319*(5862), 459-462.

Clutton-Brock, T. H. (1989). Review lecture: mammalian mating systems. *Proceedings of the Royal Society of London. B. Biological Sciences*, *236*(1285), 339-372.

Cordero, P. J., Wetton, J. H., & Parkin, D. T. (1999). Extra-pair paternity and male badge size in the house sparrow. *Journal of Avian Biology*, *30*(1), 97-102.

Crouch, N. M., & Mason-Gamer, R. J. (2018). Structural equation modeling as a tool to investigate correlates of extra-pair paternity in birds. *PloS one*, *13*(2), e0193365.

Danielsen, S. M. (2017). *Effects of perceived predation risk on extra-pair mating in blue tits (Cyanistes caeruleus)* (Master's thesis, The University of Bergen).

Dawson, D. A., Horsburgh, G. J., Küpper, C., Stewart, I. R., Ball, A. D., Durrant, K. L., ... & Krupa, A. P. (2010). New methods to identify conserved microsatellite loci and develop primer sets of high cross-species utility–as demonstrated for birds. *Molecular Ecology Resources*, *10*(3), 475-494.

Dender, M. G., Capelle, P. M., Love, O. P., Heath, D. D., Heath, J. W., & Semeniuk, C. A. (2018). Phenotypic integration of behavioural and physiological traits is related to variation in growth among stocks of Chinook salmon. *Canadian Journal of Fisheries and Aquatic Sciences*, *75*(12), 2271-2279.

Diniz, P., Macedo, R. H., & Webster, M. S. (2019). Duetting correlates with territory quality and reproductive success in a suboscine bird with low extra-pair paternity. *The Auk: Ornithological Advances*, *136*(1), uky004.

Doucet, S. M., Mennill, D. J., Montgomerie, R., Boag, P. T., & Ratcliffe, L. M. (2005). Achromatic plumage reflectance predicts reproductive success in male black-capped chickadees. *Behavioral Ecology*, *16*(1), 218-222.

Emlen, S. T., & Oring, L. W. (1977). Ecology, sexual selection, and the evolution of mating systems. *Science*, *197*(4300), 215-223.

Feeney, W. E., & Riehl, C. (2019). Monogamy without parental care? Social and genetic mating systems of avian brood parasites. *Philosophical Transactions of the Royal Society B*, *374*(1769), 20180201.

Garamszegi, L. Z., Eens, M., Hurtrez-Boussès, S., & Møller, A. P. (2005). Testosterone, testes size, and mating success in birds: a comparative study. *Hormones and Behavior*, *47*(4), 389-409.

Gerlach, N. M., McGlothlin, J. W., Parker, P. G., & Ketterson, E. D. (2012). Promiscuous mating produces offspring with higher lifetime fitness. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1730), 860-866.

Gibbs, H. L., Tabak, L. M., & Hobson, K. (1999). Characterization of microsatellite DNA loci for a neotropical migrant songbird, the Swainson's thrush (*Catharus ustulatus*). *Molecular Ecology*, *8*, 1551-1551.

Gil, D., Slater, P. J., & Graves, J. A. (2007). Extra-pair paternity and song characteristics in the willow warbler *Phylloscopus trochilus*. *Journal of Avian Biology*, *38*(3), 291-297.

Gladbach, A., Gladbach, D. J., & Quillfeldt, P. (2011). Male achromatic wing colouration is related to body condition and female reproductive investment in a dichromatic species, the upland goose. *Journal of Ethology*, *29*(2), 243-249.

Griffith, S. C., Owens, I. P., & Thuman, K. A. (2002). Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology*, *11*(11), 2195-2212.

Griggio, M., Valera, F., Casas-Crivillé, A., Hoi, H., & Barbosa, A. (2011). White tail markings are an indicator of quality and affect mate preference in rock sparrows. *Behavioral Ecology and Sociobiology*, *65*(4), 655-664.

Guindre-Parker, S. (2012). Multiple achromatic plumage signals of male quality in the snow bunting (*Plectrophenax nivalis*).

Guindre-Parker, S., Gilchrist, H. G., Baldo, S., Doucet, S. M., & Love, O. P. (2013a). Multiple achromatic plumage ornaments signal to multiple receivers. *Behavioral Ecology*, *24*(3), 672-682.

Guindre-Parker, S., & Love, O. P. (2014). Revisiting the condition-dependence of melanin-based plumage. *Journal of Avian Biology*, *45*(1), 29-33.

Guindre-Parker, S., Baldo, S., Gilchrist, H. G., Macdonald, C. A., Harris, C. M., & Love, O. P. (2013c). The oxidative costs of territory quality and offspring provisioning. *Journal of Evolutionary Biology*, *26*(12), 2558-2565.

Guindre-Parker, S., Gilchrist, H. G., Baldo, S., & Love, O. P. (2013b). Alula size signals male condition and predicts reproductive performance in an Arctic-breeding passerine. *Journal of Avian Biology*, *44*(3), 209-215.

Hill, C. E., Akçay, Ç., Campbell, S. E., & Beecher, M. D. (2011). Extrapair paternity, song, and genetic quality in song sparrows. *Behavioral Ecology*, *22*(1), 73-81.

Hoset, K. S., Espmark, Y. N. G. V. E., Moksnes, A. R. N. E., Haugan, T., Ingebrigtsen, M., & Lier, M. (2004). Effect of ambient temperature on food provisioning and reproductive success in snow buntings *Plectrophenax nivalis* in the high arctic. *Ardea*, *92*(2), 239-246.

Hoset, K. S., Espmark, Y., Fossøy, F., Stokke, B. G., Jensen, H., Wedege, M. I., & Moksnes, A. (2014). Extra-pair paternity in relation to regional and local climate in an Arctic-breeding passerine. *Polar Biology*, *37*(1), 89-97.

Jennions, M. D., & Petrie, M. (1997). Variation in mate choice and mating preferences: a review of causes and consequences. *Biological Reviews*, *72*(2), 283-327.

Johnson, K., & Burley, N. T. (1998). Mating tactics and mating systems of birds. *Ornithological Monographs*, 21-60.

Jones, A. G., Small, C. M., Paczolt, K. A., & Ratterman, N. L. (2010). A practical guide to methods of parentage analysis. *Molecular Ecology Resources*, *10*(1), 6-30.

Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, *16*(5), 1099-1106.

Kallinowski, S. T., Taper, M. L., & Marshall, T. C. (2010). Corrigendum. *Molecular Ecology*, *19*, 1512.

Kempenaers, B., Verheyen, G. R., & Dhondi, A. A. (1997). Extrapair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics, and offspring quality. *Behavioral Ecology*, *8*(5), 481-492.

Kleven, O., Marthinsen, G., & Lifjeld, J. T. (2006). Male extraterritorial forays, age and paternity in the socially monogamous reed bunting (*Emberiza schoeniclus*). *Journal of Ornithology*, *147*(3), 468-473.

Kvarnemo, C. (2018). Why do some animals mate with one partner rather than many? A review of causes and consequences of monogamy. *Biological Reviews*, *93*(4), 1795-1812.

Lack, D. L. (1968). Ecological adaptations for breeding in birds.

Lebigre, C., Arcese, P., Sardell, R. J., Keller, L. F., & Reid, J. M. (2012). Extra-pair paternity and the variance in male fitness in song sparrows (*Melospiza melodia*). *Evolution: International Journal of Organic Evolution*, *66*(10), 3111-3129.

Lehtonen, P. K., Primmer, C. R., & Laaksonen, T. (2009). Different traits affect gain of extrapair paternity and loss of paternity in the pied flycatcher, *Ficedula hypoleuca*. *Animal Behaviour*, *77*(5), 1103-1110.

Lifjeld, J. T., Laskemoen, T., Kleven, O., Albrecht, T., & Robertson, R. J. (2010). Sperm length variation as a predictor of extrapair paternity in passerine birds. *PLoS One*, *5*(10), e13456.

Macdonald, C. A., McKinnon, E. A., Gilchrist, H. G., & Love, O. P. (2016). Cold tolerance, and not earlier arrival on breeding grounds, explains why males winter further north in an Arctic-breeding songbird. *Journal of Avian Biology*, *47*(1), 7-15.

Macedo, R. H., Karubian, J., & Webster, M. S. (2008). Extrapair paternity and sexual selection in socially monogamous birds: are tropical birds different?. *The Auk*, *125*(4), 769-777.

Marshall, T. C., Slate, J. B. K. E., Kruuk, L. E. B., & Pemberton, J. M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, *7*(5), 639-655.

McGlothlin, J. W., Duffy, D. L., Henry-Freeman, J. L., & Ketterson, E. D. (2007). Diet quality affects an attractive white plumage pattern in dark-eyed juncos (*Junco hyemalis*). *Behavioral Ecology and Sociobiology*, *61*(9), 1391-1399.

McKinnon, E. A., Macdonald, C. M., Gilchrist, H. G., & Love, O. P. (2016). Spring and fall migration phenology of an Arctic-breeding passerine. *Journal of Ornithology*, *157*(3), 681-693.

Møller, A. P. (2000). Male parental care, female reproductive success, and extrapair paternity. *Behavioral Ecology*, *11*(2), 161-168.

Montgomerie, R.D. & Lyon, B.E. (2011). Snow bunting (*Plectrophenax nivalis*). The birds of North America online. (A. Poole Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from Birds of North America Online: http://bna.birds.cornell.edu/bna/species/19

O'Brien, E. L., & Dawson, R. D. (2011). Plumage color and food availability affect male reproductive success in a socially monogamous bird. *Behavioral Ecology*, *22*(1), 66-72.

Oliveira, D. P., Marioni, B., Farias, I. P., & Hrbek, T. (2014). Genetic evidence for polygamy as a mating strategy in *Caiman crocodilus*. *Journal of Heredity*, *105*(4), 485-492.

Orians, G. H. (1969). On the evolution of mating systems in birds and mammals. *The American Naturalist*, *103*(934), 589-603.

Owens, I. P., & Short, R. V. (1995). Hormonal basis of sexual dimorphism in birds: implications for new theories of sexual selection. *Trends in Ecology & Evolution*, *10*(1), 44-47.

Piertney, S. B., Marquiss, M., & Summers, R. (1998). Characterization of tetranucleotide microsatellite markers in the Scottish crossbill (*Loxia scotica*). *Molecular Ecology*, *7*(9), 1261-1263.

Poesel, A., Gibbs, H. L., & Nelson, D. A. (2011). Extrapair fertilizations and the potential for sexual selection in a socially monogamous songbird. *The Auk*, *128*(4), 770-776.

Raouf, S. A., Parker, P. G., Ketterson, E. D., Nolan Jr, V., & Ziegenfus, C. (1997). Testosterone affects reproductive success by influencing extra–pair fertilizations in male dark–eyed juncos (Aves: *Junco hyemalis*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *264*(1388), 1599-1603.

Richardson, D. S., & Burke, T. (2001). Extrapair paternity and variance in reproductive success related to breeding density in Bullock's orioles. *Animal Behaviour*, *62*(3), 519-525.

Roberts, M. L., Buchanan, K. L., & Evans, M. R. (2004). Testing the immunocompetence handicap hypothesis: a review of the evidence. *Animal Behaviour*, *68*(2), 227-239.

Schlicht, E., & Kempenaers, B. (2011). Extra-pair paternity and sexual selection. In *From genes to animal behavior* (pp. 35-65). Springer, Tokyo.

Sefc, K. M., Payne, R. B., & Sorenson, M. D. (2001). Characterization of microsatellite loci in village indigobirds *Vidua chalybeata* and cross-species amplification in estrildid and ploceid finches. *Molecular Ecology Notes*, *1*(4), 252-254.

Shuster, S. M., & Wade, M. J. (2019). *Mating systems and strategies*. Princeton University Press.

Siitari, H., & Huhta, E. (2002). Individual color variation and male quality in pied flycatchers (*Ficedula hypoleuca*): a role of ultraviolet reflectance. *Behavioral Ecology*, *13*(6), 737-741.

Sinervo, B., Chaine, A. S., & Miles, D. B. (2020). Social Games and Genic Selection Drive Mammalian Mating System Evolution and Speciation. *The American Naturalist*, *195*(2), 247-274.

Slate, J., Marshall, T., & Pemberton, J. (2000). A retrospective assessment of the accuracy of the paternity inference program CERVUS. *Molecular Ecology*, *9*(6), 801-808.

Stewart, S. L., Westneat, D. F., & Ritchison, G. (2010). Extra-pair paternity in eastern bluebirds: effects of manipulated density and natural patterns of breeding synchrony. *Behavioral Ecology and Sociobiology*, *64*(3), 463-473.

Stutchbury, B. J., & Morton, E. S. (1995). The effect of breeding synchrony on extra-pair mating systems in songbirds. *Behaviour*, 675-690.

Stutchbury, B. J., & Morton, E. S. (2001). *Behavioral Ecology of tropical birds*. Academic press, San Diego, USA.

Thusius, K. J., Peterson, K. A., Dunn, P. O., & Whittingham, L. A. (2001). Male mask size is correlated with mating success in the common yellowthroat. *Animal Behaviour*, *62*(3), 435-446.

Webster, M. S., Varian, C. W., & Karubian, J. (2008). Plumage color and reproduction in the Red-backed Fairy-wren: why be a dull breeder?. *Behavioral Ecology*, *19*(3), 517-524.

Wells, S. J., Ji, W., Dale, J., Jones, B., & Gleeson, D. (2015). Male size predicts extrapair paternity in a socially monogamous bird with extreme sexual size dimorphism. *Behavioral Ecology*, *26*(1), 200-206.

Westneat, D. F., & Stewart, I. R. (2003). Extra-pair paternity in birds: causes, correlates, and conflict. *Annual Review of Ecology, Evolution, and Systematics*, *34*(1), 365-396.

Whittingham, L. A., & Dunn, P. O. (2005). Effects of extra-pair and within-pair reproductive success on the opportunity for selection in birds. *Behavioral Ecology*, *16*(1), 138-144.

Wingfield, J. C. (1984). Androgens and mating systems: testosterone-induced polygyny in normally monogamous birds. *The Auk*, *101*(4), 665-671.

Wink, M., & Dyrcz, A. (1999). *Mating systems in birds: a review of molecular studies*. Muzeum i Instytut Zoologii PAN.

Wittenberger, J. F. (1979). The evolution of mating systems in birds and mammals. In *Social behavior and communication* (pp. 271-349). Springer, Boston, MA.

Wonke, G., Wallschläger, D., Moll, K., & Tiedemann, R. (2007). Ten new microsatellite loci for the yellowhammer (*Emberiza citrinella*) and their cross-species applicability among related taxa. *Molecular Ecology Notes*, *7*(6), 1278-1280.

Zanollo, V., Griggio, M., Robertson, J., & Kleindorfer, S. (2012). The number and coloration of white flank spots predict the strength of a cutaneous immune response in female Diamond Firetails, *Stagonopleura guttata*. *Journal of Ornithology*, *153*(4), 1233-1244.

Tables

Table 3.1: Predicted impact of male quality traits on components of realized fitness (within-pair reproductive success: WPRS & extra-pair reproductive success: EPRS) in snow buntings (*Plectrophenax nivalis*). Detailed trait descriptions are provided in Appendix B1, and rationales behind our choice are provided in 'Methods'.

Category	**Male quality trait**	**Predicted directional relationship**	
		WPRS	EPRS
Male State	Arrival body mass	-	+
	Arrival plasma testosterone	+	-
	Arrival oxidative status	-	+
	Arrival plasma immunoglobulin Y	-	+
Song Quality	Note duration	-	+
	Song length	-	+
	Syllable repetition	-	+
	Song versatility	-	+
Wing Pattern	Area of spots	+	-
	Average spot size	+	-
	Area of extremity	+	-
	Area of alula	-	+
Body Plumage	Breast brightness	-	+
	Breast UV chroma	-	+
	Breast saturation	-	+
	Mantle brightness	-	+
	Mantle UV chroma	-	+
	Mantle saturation	-	+
Territory Quality	Territory size	+	-
	Rock cover	-	+

Table 3.2: Parameters for the nine microsatellite markers used for paternity analysis for snow buntings (*Plectrophenax nivalis*) located at Mitivik Island, Nunavut, Canada in 2010 and 2011.

Locus	k	H_{Obs}	H_{Exp}	PIC	NE-1P	NE-2P	NE-PP	HW	F(Null)
Year: 2010									
SNBU682	9	0.479	0.503	0.481	0.855	0.682	0.488	NS	0.0296
CUU28	9	0.65	0.763	0.722	0.635	0.457	0.272	NS	0.0783
INDIGO29	20	0.704	0.921	0.91	0.29	0.169	0.047	ND	0.1292
SNBU705	22	0.559	0.918	0.907	0.299	0.175	0.05	ND	0.2436
CAM17	4	0.591	0.616	0.537	0.809	0.671	0.521	NS	0.0147
ECIT2	8	0.736	0.691	0.645	0.718	0.542	0.351	NS	-0.0383
POCC6	8	0.495	0.549	0.513	0.832	0.664	0.477	NS	0.0372
LOX8	17	0.627	0.898	0.884	0.353	0.214	0.071	ND	0.1706
GF12	16	0.927	0.923	0.912	0.286	0.167	0.046	ND	-0.0054
Year: 2011									
SNBU682	9	0.597	0.616	0.589	0.77	0.583	0.372	NS	0.0036
CUU28	9	0.627	0.73	0.678	0.687	0.514	0.332	NS	0.0781
INDIGO29	15	0.863	0.896	0.88	0.364	0.222	0.076	ND	0.0141
SNBU705	20	0.761	0.911	0.897	0.317	0.189	0.055	ND	0.0887
CAM17	3	0.547	0.588	0.5	0.829	0.704	0.562	NS	0.0283
ECIT2	8	0.707	0.698	0.653	0.707	0.531	0.337	NS	-0.0031
POCC6	6	0.575	0.614	0.541	0.801	0.657	0.494	NS	0.0353
LOX8	17	0.69	0.915	0.902	0.309	0.183	0.053	ND	0.1355
GF12	18	0.925	0.909	0.892	0.333	0.2	0.063	ND	-0.0149

Locus: Microsatellite marker name; k: # of alleles; HObs: Observed heterozygosity; HExp: Expected heterozygosity; PIC: Polymorphic information content; NE-1P: Average non-exclusion probability for the mother; NE-2P: Average non-exclusion probability for the father given the genotype of the mother; NE-PP: Average non-exclusion probability for a candidate parent pair; HW: Hardy Weinberg Equilibrium test, NS = not significant; F(Null): Estimated null allele frequency. The combined probability of parental exclusion was 0.999.

Table 3.3: Rates of extra-pair paternity among snow bunting (*Plectrophenax nivalis*) broods and nestlings across 2010 and 2011 at located at Mitivik Island, Nunavut, Canada.

	Broods		**Nestlings**	
Year	**# analyzed**	**# containing EPY (% ± SE)**	**# analyzed**	**# of EPY (% ± SE)**
2010	16	12 (75.0 ± 10.8)	84	30 (35.7 ± 5.2)
2011	13	7 (53.9 ± 13.8)	50	21 (42.0 ± 7.0)

Table 3.4: Generalized Linear Models (GLMs) examining links between male quality trait and three measures of reproductive success in male snow buntings *(Plectrophenax nivalis)* breeding at Mitivik Island, Nunavut, Canada.

Model 1: Within-Pair Reproductive Success (WPRS)		**LogWorth**	**Chi-Square**	**P-Value**
Overall model			9.33	0.230
Over-dispersion			11.48	0.244
Independent variables:	Arrival date	0.12	0.10	0.754
	Number of male neighbours	0.35	0.57	0.450
	Brood size	2.28	7.78	**0.005**
	Combined PC1	0.60	1.33	0.249
	Combined PC2	0.75	1.80	0.180
	Combined PC3	0.05	0.02	0.882
	Song Complexity PC	0.16	0.15	0.701
Model 2: Extra-Pair Reproductive Success (EPRS)		LogWorth	Chi-Square	P-Value
Overall model			19.09	**0.008**
Over-dispersion			13.68	0.134
Independent variables:	Arrival date	0.79	1.96	0.161
	Number of male neighbours	0.15	0.13	0.714
	WPRS	2.01	6.68	**0.010**
	Combined PC1	0.08	0.04	0.841
	Combined PC2	3.04	11.01	**0.001**
	Combined PC3	0.73	1.76	0.185
	Song Complexity PC	0.11	0.08	0.781
Model 3: EPRS Allocation		LogWorth	Chi-Square	P-Value
Overall model			10.62	0.156
Over-dispersion			12.63	0.180
Independent variables:	Arrival date	0.31	0.48	0.489
	Number of male neighbours	0.06	0.03	0.873
	WPRS	1.24	3.61	**0.057***
	Combined PC1	0.04	0.01	0.905
	Combined PC2	1.46	4.45	**0.035**
	Combined PC3	0.41	0.73	0.392
	Song Complexity PC	0.49	0.96	0.328

*** Significant at alpha level 0.1**

Figures

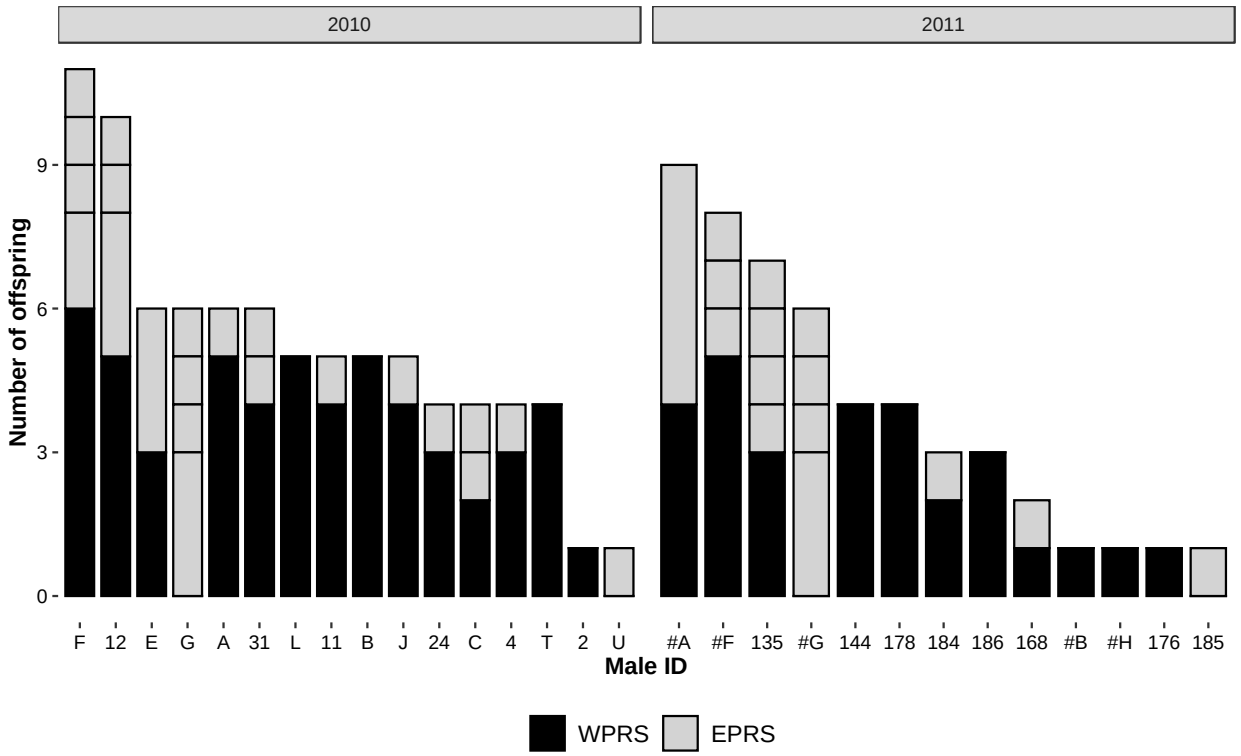


Figure 3.1: Within-pair and extra-pair reproductive success (WPRS and EPRS, respectively) of male snow buntings (*Plectrophenax nivalis*) breeding at Mitivik Island, Nunavut, Canada in 2010 and 2011. Further divisions within EPRS bars indicate EPRS allocation across multiple nests. Individuals L & #A, and U & #H are identical, respectively, as they were repeat-breeders across years.

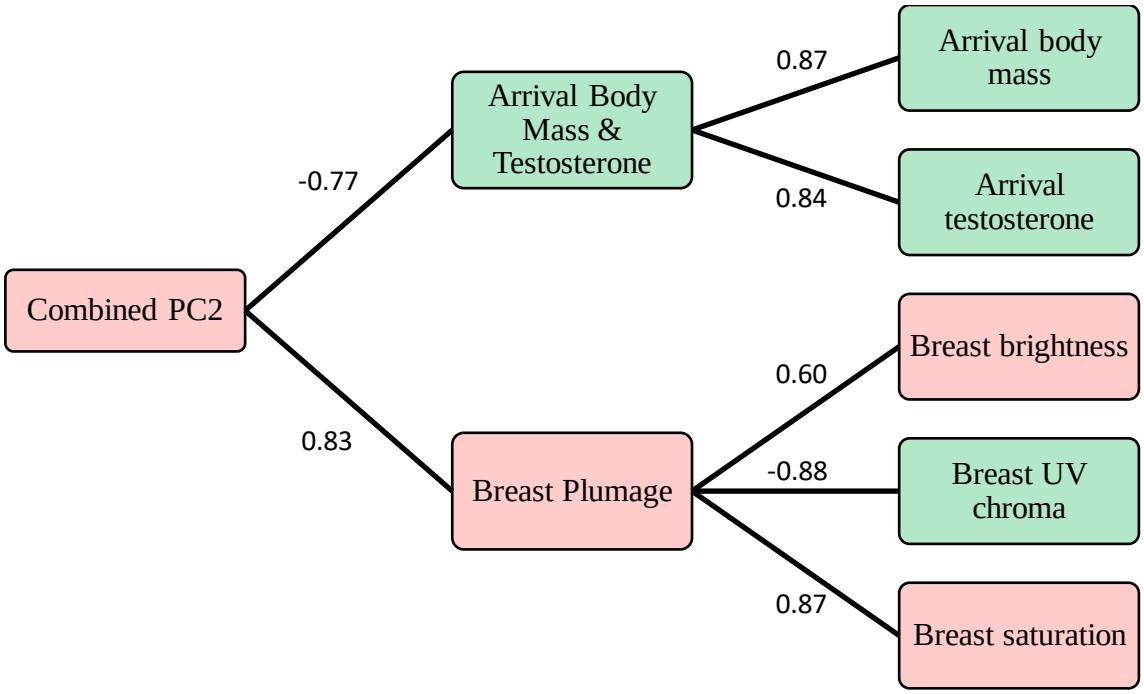


Figure 3.2: Visual representation of directional relationships between male quality traits (from Generalized Linear Models (GLMs)) and variation in Extra-pair Reproductive Success (EPRS) for male snow buntings (*Plectrophenax nivalis*) breeding at Mitivik Island, Nunavut, Canada. Green and red represent positive and negative relationships, respectively, the values indicate PCA loadings, and all relationships shown are significant at alpha level 0.05.

CHAPTER 4

GENERAL DISCUSSION

The effects of climate change are most severely felt in the Arctic (Canosa et al., 2020; Wauchope et al., 2017), and are associated with diverse and substantial ecosystem disturbances. As such, many Arctic-migratory species are experiencing population decline and are at risk of extirpation as climate change is expected to accelerate (IPCC 2021). Consequently, dramatic alteration of local environmental conditions is expected to impact the survival and reproduction of a diversity of Arctic species (Box et al., 2019; Malhi et al., 2020). However, the integration of molecular genetic technology, ecology and evolutionary biology could provide transformative insights into the management and conservation of Arctic biodiversity (Sauve et al., 2019; Peeters et al., 2020). While standing genetic diversity has been shown to play a role in adaptive potential in a variety of species (Barrett & Schluter, 2008), it is generally not included in species management decisions (Laikre et al., 2010; Coates et al., 2018) as genetic diversity is assumed to be of minor concern compared to other more pressing factors (Cutter & Payseur, 2013). Population persistence depends on the processes that govern the survival and reproduction of individuals, therefore consideration of local and global genetic diversity and predictors of variation in reproductive success are crucial for the success of any management plan (Colella et al., 2020). Given the logistical challenges to monitoring Arctic-breeding populations, genetic analyses provide a robust, minimally invasive approach to how and why individuals vary over time, both among and within populations (Layton et al., 2021).

This thesis contributes to our knowledge of factors driving local adaptation and variation in male reproductive success in breeding populations of snow bunting (*Plectrophenax nivalis*). In Chapter 2, I used a combination of population genetic and candidate gene polymorphism analyses to demonstrate reproductive isolation, genetic population structure, and local adaptation among six, globally distributed, breeding snow bunting populations. The observed patterns of divergence were explained by both genetic drift and selection at functional genetic markers, suggesting a role of demographic processes as well as natural selection in the structuring of breeding populations. In Chapter 3, I identified key male quality traits as drivers of variation in male extra-pair reproductive fitness (EPRS), and as extra-pair paternity (EPP) increases the overall reproductive fitness of high-quality males, my work highlights the potential role of sexual selection on male phenotypic traits in snow buntings. Here I discuss the key results of my thesis, provide interpretations, and explore their implications in connection to the conservation and management of this widely-distributed Arctic-breeding passerine. I also extend the discussion to Arctic-migratory birds in general.

One particularly interesting finding from my thesis was the pattern of reproductive isolation among snow bunting breeding populations, which is generally unexpected among long-distance migratory species (Arguedas & Parker, 2000; Winker et al., 2000). This finding is important because conservation efforts for migratory species are generally hindered by limited knowledge of species distribution, abundance, genetic structure, and potentially adaptive traits (Schuster et al., 2019; Zuckerberg et al., 2016). Although the general distributions of snow bunting breeding and wintering grounds are well defined (Montgomerie & Lyon, 2020), abundance estimates are only known for

North American populations, and even those have shown significant declines in the last few decades (Butcher & Niven, 2007). It is highly likely that these trends are global, and the characterizing genetic structure and adaptive traits, both of which were scarce in the snow bunting literature, will aid in designing specific management strategies for different breeding populations. Such interventions can now be designed to maximize retention of functionally adaptive traits to maintain global and local genetic diversity in the face of population decline (Moritz, 2002; Hoffman, 2010). Captive breeding programs operate with a primary goal of maintaining or even increasing genetic diversity by breeding genetically dissimilar individuals (Willi et al., 2021) to counteract local population declines and potential extirpations. However, such an approach may not be advisable when dealing with locally adapted populations as this can result in outbreeding depression (Hendry et al., 2000), worsening the problem. This may be of particular relevance for snow buntings, as the majority of selection signatures I observed were of stabilizing nature, suggesting high levels of functional similarities across populations, which may entice conservation practitioners to translocate individuals to supplement declining populations.

Although the global perspective of my thesis provided evidence for connectivity and isolation among breeding populations (Chapter 2), the local perspective was valuable in assessing mate choice strategies that directly affect fitness at an individual level (Chapter 3). Given the potential local effects of adaptation among breeding populations, it is possible that individual reproductive behavioral decisions may be habitat-specific (Quader, 2005), and may thus change in the context of environmental change within a population. More importantly, since I showed evidence of population structure globally

(Chapter 2), our findings on differential male reproductive strategies (Chapter 3) may not be consistent in other breeding populations. Additionally, if there is adaptive plasticity in individual reproduction-related behaviours (i.e., mate choice preferences, altering migration patterns, etc.), it will likely alter the global genetic diversity and population structure.

One of the remarkable findings from Chapter 3 was that some male quality traits positively predicted EPRS, yet no traits appeared to predict variation in within-pair reproductive success (WPRS), suggesting differential mechanisms driving mate choice in females. This finding was interesting at first as it suggests that assessment of male quality is not included in a female's choice of choosing a social (within-pair) mate. Therefore, a closer examination to assess links between WPRS and male quality (if it is indeed present) should be addressed by future studies perhaps using similar male quality trait data but assessing it with larger sample size and in populations with lower breeding densities than the one studied here. Given that such links may have been undetectable in our study due to high breeding density driving EPP levels (Chapter 3), and hence overwhelming our correlations between EPRS and male quality, a related project, but with more typical breeding density is indicated. If such relationships do not exist after further investigation across populations, then it suggests that either the evolution of male quality traits solely depends on female choice associated with traits involved in EPRS, or that within-pair mate choices by females are linked to male traits that I have not yet measured here (e.g., male traits that affect offspring quality or survival rather than simply number of offspring – see 'Future directions' section below). Additionally, I speculate that female mate choice may be a key driver of divergent selection at genes involved in

various aspects of reproduction (candidate genes from Chapter 2), which should be an interesting future approach based on my existing results. An understanding of the linkages between male quality traits and female preferences and their potential to increase population differentiation would help conservation agencies identify factors that could impact the success of possible interventions (Asa et al., 2011). For example, female preference for male quality would clearly need to be incorporated into designing possible captive breeding strategies (Sun et al., 2019), which has not been considered for migratory birds to the best of our knowledge, but is widely discussed in mammals (e.g. Stripe-faced dunnart (*Smithopsis macroura*), Parrot et al., 2019; the koala (*Phascolarctos cinereus*), Brandies et al., 2018, among others), and fish (e.g., Atlantic Salmon (*Salmo salar*), Consuegra & Garcia de Leaniz, 2008; Coho Salmon (*Oncorhynchus kisutch*), Auld et al., 2021, among others).

This thesis has opened multiple avenues for further research on snow buntings and Arctic-migratory birds (discussed under 'Future directions' below). In the short-term, I suggest following approaches using our current results and data collected throughout the two data chapters: i) exploring the function and type of variants for functional genes identified as being under divergent selection among population pairs, ii) quantifying migration rates between the Alert and Mitivik Island, and Barrow and Svalbard breeding populations, iii) constructing a visual spatial network of male breeding behaviours and subsequent EPP investment using a map of the Mitivik Island sampling site, and iv) assessing pairwise differences in male quality for social male and extra-pair male for each female. Revisiting these studies as a baseline measure to assess the impact of climate stressors on locally adapted traits and reproductive behaviours would forge a path

to effective on-going monitoring and management of this Arctic-breeding passerines, as well as other species facing similar environmental challenges.

Limitations, improvements and future directions

Experimental and sampling improvements

The first goal of this study was to assess population structure across as much of the snow bunting breeding range as currently possible (Chapter 2). Based on the current sample set, I was able to demonstrate spatial genetic structure (i.e., across six populations), but I did not detect significant temporal genetic variation. As changing environmental conditions have the potential to alter population structure and drive adaptive population divergence across space *and* time (Hereford, 2009) it is important to consider temporal genetic variation as well. While it may be difficult to fill past sampling gaps (i.e., using museum specimens which may provide low sample size or degraded tissues for genetic analyses; see Raxworthy, 2021) for snow buntings, future sampling of the populations included in this study through the established network of researchers will allow ongoing monitoring of the populations included in our study. I suggest that ideally as many other breeding populations across the Holarctic breeding range as possible should be added to the baseline genetic dataset.

A second major goal of this thesis was to assess relationship between male quality traits and variation in reproductive success (Chapter 3). My results are correlational, hence I propose that future controlled experiments would be valuable to assess the direct link of cause and effect between male quality traits and variation in EPRS of males. It may be possible to manipulate individual male quality traits in the wild (i.e., clipping feathers to alter apparent plumage quality, testosterone level manipulation through

implants, etc.). However, such a project could be challenging, especially since I examined male quality as a whole (i.e., multiple phenotypic measures) rather than each trait individually so that manipulating individual male traits through such studies may not reflect the complexity of the relationship. Additionally, isolated manipulative experiments may not be relevant to wild populations as female choice preferences can vary inter-annually (e.g., Chaine and Lyon, 2008), likely due to associated changes in environmental conditions (Burley & Foster, 2006). Although I worked with two years of male quality data, I did not include year as a covariate in our final three models as it consistently did not show as a significant effect and was removed from the final models. However, testing for temporal effects over two successive years cannot capture the range of possible temporal effects on female choice. I thus propose a multi-year approach to increase the temporal scale of future studies to assess potential inter-annual adaptive plasticity in female choice and male reproductive behaviours.

Future directions

This thesis focussed on breeding populations of snow buntings, as individuals are expected to face the strongest selective pressures during the critical breeding period, allowing us to assess factors contributing to local adaptation (Chapter 2) and variation in reproductive success (Chapter 3). A key limitation in our understanding of snow bunting ecology is unidentified wintering grounds and their important connectivity link to breeding populations, as this information is currently only known for individuals breeding at Mitivik Island and Svalbard (Macdonald et al., 2012, Snell et al., 2018). Since the genetic markers I used in Chapter 2 identified potential geneflow between Alert and Mitivik Island, and Barrow and Svalbard, future studies should explore the migratory

connectivity through tracking studies for Alert and Barrow individuals. Since natural selection acts directly on phenotypes, and not genotypes (Brandon, 1982), future research should explore the role of gene transcription regulation in local adaptation though investigating population-level gene-expression differences at candidate loci under divergent selection (Chapter 2). This would be especially relevant for genes that are involved in various aspects of reproduction as potential differences in individual gene expression may explain genotypic basis to individual variation in reproductive fitness within a population (quantified in Chapter 3). Furthermore, future work should consider additional fitness-related metrics (i.e., offspring quality, survival, and future reproductive success) related to within-pair female mate choice that go beyond measuring the number of offspring to explore the role of male quality and female choice in WPRS variation. Finally, future studies could conduct reciprocal transplant experiments to assess direct fitness consequences of identified genes (Chapter 2) and traits of interest to reinforce the link between local adaptation and reproductive fitness.

Summary

In conclusion, my thesis provides evidence for substantial population differentiation driven by selection and drift, as well as variation in male reproductive success. Accelerated environmental change in the Arctic demands recognition, management and on-going monitoring of biodiversity using fine-scale genetic approaches. I emphasize prioritizing maintenance of standing genetic variation in local populations, and understanding flexibility in reproductive behavior, which maximizes adaptive capacity of species. As long-distance migratory birds typically travel over large distances, the responsibility for their conservation and management must be shared internationally.

Conservation of such long-distance migratory bird will not be simple; however, I hope my findings will help direct such efforts.

References

Asa, C. S., Traylor-Holzer, K., & Lacy, R. C. (2011). Can conservation-breeding programmes be improved by incorporating mate choice?. *International Zoo Yearbook, 45*(1), 203-212.

Auld, H. L., Jacobson, D. P., Rhodes, A. C., & Banks, M. A. (2021). Differences in Mate Pairings of Hatchery-and Natural-Origin Coho Salmon Inferred from Offspring Genotypes. *Integrative Organismal Biology, 3*(1), obab020.

Barrett, R. D., & Schluter, D. (2008). Adaptation from standing genetic variation. *Trends in Ecology & Evolution, 23*(1), 38-44.

Box, J. E., Colgan, W. T., Christensen, T. R., Schmidt, N. M., Lund, M., Parmentier, F. J. W., ... & Olsen, M. S. (2019). Key indicators of Arctic climate change: 1971–2017. *Environmental Research Letters, 14*(4), 045010.

Brandies, P. A., Grueber, C. E., Ivy, J. A., Hogg, C. J., & Belov, K. (2018). Disentangling the mechanisms of mate choice in a captive koala population. *PeerJ, 6*, e5438.

Brandon, R. (1982, January). The levels of selection. In *PSA: Proceedings of the biennial meeting of the Philosophy of Science Association* (Vol. 1982, No. 1, pp. 315-323). Philosophy of Science Association.

Burley, N. T., & Foster, V. S. (2006). Variation in female choice of mates: condition influences selectivity. *Animal Behaviour, 72*(3), 713-719.

Butcher, G. S., & Niven, D. K. (2007). Combining data from the Christmas Bird Count and the Breeding Bird Survey to determine the continental status and trends of North America birds.

Canosa, I. V., Ford, J. D., Mcdowell, G., Jones, J., & Pearce, T. (2020). Progress in climate change adaptation in the Arctic. *Environmental Research Letters, 15*(9), 093009.

Chaine, A. S., & Lyon, B. E. (2008). Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the lark bunting. *Science, 319*(5862), 459-462.

Colella, J. P., Talbot, S. L., Brochmann, C., Taylor, E. B., Hoberg, E. P., & Cook, J. A. (2020). Conservation genomics in a changing Arctic. *Trends in Ecology & Evolution, 35*(2), 149-162.

Consuegra, S., & Garcia de Leaniz, C. (2008). MHC-mediated mate choice increases parasite resistance in salmon. *Proceedings of the Royal Society B: Biological Sciences, 275*(1641), 1397-1403.

Hendry, A. P., Wenburg, J. K., Bentzen, P., Volk, E. C., & Quinn, T. P. (2000). Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. *Science, 290*(5491), 516-518.

Hereford, J. (2009). A quantitative survey of local adaptation and fitness trade-offs. *The American Naturalist, 173*(5), 579-588.

Hoffmann, I. (2010). Climate change and the characterization, breeding and conservation of animal genetic resources. *Animal genetics, 41*, 32-46.

IPCC, 2021: Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University Press. In Press.

Layton, K. K. S., Snelgrove, P. V. R., Dempson, J. B., Kess, T., Lehnert, S. J., Bentzen, P., ... & Bradbury, I. R. (2021). Genomic evidence of past and future climate-linked loss in a migratory Arctic fish. *Nature Climate Change, 11*(2), 158-165.

Macdonald, C. A., Fraser, K. C., Gilchrist, H. G., Kyser, T. K., Fox, J. W., & Love, O. P. (2012). Strong migratory connectivity in a declining Arctic passerine. *Animal Migration, 1*(1), 23-30.

Malhi, Y., Franklin, J., Seddon, N., Solan, M., Turner, M. G., Field, C. B., & Knowlton, N. (2020). Climate change and ecosystems: Threats, opportunities and solutions. *Philosophical Transactions of the Royal Society B, 375*(1794), 20190104.

Montgomerie, R. and B. Lyon (2020). Snow Bunting (*Plectrophenax nivalis*), version 1.0. In Birds of the World (S. M. Billerman, B. K. Keeney, P. G. Rodewald, and T. S. Schulenberg, Editors). Cornell Lab of Ornithology, Ithaca, NY, USA. https://doi-org.ledproxy2.uwindsor.ca/10.2173/bow.snobun.01

Moritz, C. (2002). Strategies to protect biological diversity and the evolutionary processes that sustain it. *Systematic biology, 51*(2), 238-254.

Parrott, M. L., Nation, A., & Selwood, L. (2019). Female mate choice significantly increases captive breeding success, and scents can be frozen to determine choice, in the stripe-faced dunnart. *Applied Animal Behaviour Science, 214*, 95-101.

Peeters, B., Le Moullec, M., Raeymaekers, J. A., Marquez, J. F., Røed, K. H., Pedersen, Å. Ø., ... & Hansen, B. B. (2020). Sea ice loss increases genetic isolation in a high Arctic ungulate metapopulation. *Global Change Biology, 26*(4), 2028-2041.

Quader, S. (2005). Mate choice and its implications for conservation and management. *Current science*, 1220-1229.

Raxworthy, C. J., & Smith, B. T. (2021). Mining museums for historical DNA: Advances and challenges in museomics. *Trends in Ecology & Evolution*, *36*(11), 1049-1060.

Sauve, D., Divoky, G., & Friesen, V. L. (2019). Phenotypic plasticity or evolutionary change? An examination of the phenological response of an arctic seabird to climate change. *Functional Ecology*, *33*(11), 2180-2190.

Schuster, R., Wilson, S., Rodewald, A. D., Arcese, P., Fink, D., Auer, T., & Bennett, J. R. (2019). Optimizing the conservation of migratory species over their full annual cycle. *Nature Communications*, *10*(1), 1-8.

Snell, K. R., Stokke, B. G., Moksnes, A., Thorup, K., & Fossøy, F. (2018). From Svalbard to Siberia: Passerines breeding in the High Arctic also endure the extreme cold of the Western Steppe. *PloS one*, *13*(9), e0202114.

Sun, L., Zhou, T., Stone, G. N., Wan, Q. H., & Fang, S. G. (2019). Seeing-good-gene-based mate choice: From genes to behavioural preferences. *Journal of Animal Ecology*, *88*(11), 1708-1719.

Wauchope, H. S., Shaw, J. D., Varpe, Ø., Lappo, E. G., Boertmann, D., Lanctot, R. B., & Fuller, R. A. (2017). Rapid climate-driven loss of breeding habitat for Arctic migratory birds. *Global Change Biology*, *23*(3), 1085-1094.

Willi, Y., Kristensen, T. N., Sgrò, C. M., Weeks, A. R., Ørsted, M., & Hoffmann, A. A. (2022). Conservation genetics as a management tool: The five best-supported paradigms to assist the management of threatened species. *Proceedings of the National Academy of Sciences*, *119*(1).

Zuckerberg, B., Fink, D., La Sorte, F. A., Hochachka, W. M., & Kelling, S. (2016). Novel seasonal land cover associations for eastern North American forest birds identified through dynamic species distribution modelling. *Diversity and Distributions*, *22*(6), 717-730.

APPENDICES

Appendix A: Supplemental material for Chapter 2

Appendix A1: RNA Sequencing and read mapping summary statistics for 16 snow bunting (*Plectrophenax nivalis*) individuals chosen for transcriptome analyses. The last two columns refer to mapping of the individual samples to the *de-novo* assembled transcriptome using BWA v0.7.12.

Sample	Number of bases	Number of reads	Average quality	% Duplicate	% Alignment	% Properly paired
SB01	8,725,385,600	43,626,928	38	13.73	98.86	90.07
SB02	9,511,951,400	47,559,757	38	14.136	98.77	90.31
SB03	8,924,712,400	44,623,562	38	13.757	98.84	90.18
SB04	9,199,309,200	45,996,546	39	14.616	98.90	90.17
SB05	9,390,082,200	46,950,411	38	14.409	98.87	90.16
SB06	8,946,775,400	44,733,877	38	13.524	98.83	90.43
SB07	8,899,469,600	44,497,348	39	13.754	98.87	90.36
SB08	7,996,530,600	39,982,653	38	12.197	98.84	89.88
SB09	10,439,844,800	52,199,224	39	15.902	98.92	90.74
SB10	9,276,897,800	46,384,489	39	14.301	98.90	91.09
SB11*	8,939,979,200	44,699,896	38	15.007	98.87	90.72
SB12	9,668,835,200	48,344,176	38	15.671	98.89	90.30
SB13	9,610,411,800	48,052,059	38	14.768	98.87	90.44
SB14	8,287,933,000	41,439,665	38	13.802	98.84	90.25
SB15*	7,424,016,600	37,120,083	38	12.816	98.85	91.03
SB16	8,892,934,000	44,464,670	39	14.673	98.90	90.55
Total	144,135,068,800	720,675,344				

*These samples were not included in *de-novo* transcriptome assembly due to limitations on computational memory.

Appendix A2: Summary statistics for *de-novo* assembled transcriptome and Single Nucleotide Polymorphism (SNP) characterization for snow bunting (*Plectrophenax nivalis*) individuals based on RNA Sequencing data.

Statistic		
Number of transcripts		534815
Total bp of transcriptome		373011802
Number of SNPs characterized		11378
Number of SNPs form a transcript with a valid start codon		9756
Variant Type		
	Upstream gene variant	1445
	Downstream gene variant	4266
	Intergenic region	2753
	Missense variant	274
	Synonymous variant	885
	Other	133

Appendix A3: Description of 117 SNP loci (with forward and reverse primer sequences 5'-3') for snow buntings (*Plectrophenax nivalis*) designed for amplification using five multiplex PCR reactions. 'Gene Description' was determined based on Gene Ontology database (Gene Ontology Consortium) and UniProt database. 'Type of Variant' for each SNP locus was annotated using SNPEff. 'Multiplex Group' refers to one of the five groups the primer belonged to for the multiplex reactions prior to Ion Torrent sequencing. 'Gene Function' refers to the following categories: Energetics, Cellular Housekeeping, Immune Response, Lipid Metabolism, Nervous System Development, Reproduction, and Stress Response. 'Transcript ID' refers to the identity of the transcript from the de-novo assembled reference transcriptome that was used to design that specific primer. Asterisk represents SNP loci (n=16) that were excluded from population genetic analyses due to low sequence data available across populations.

SNP Primer Name	Gene Description	Type of Variant	Nucleotide Variant	Amino acid Variant	Multiplex Group	Forward Primer	Reverse Primer	Transcript ID
Gene Function: Energetics (10 SNPs)								
SNP_125	Sodium channel subunit beta-2	Downstream	G/A		5	TGCTTCCAGAGCAAGGATTT	TACACTGGCACAACCCAGAG	TRINITY_DN11049_c0_g1
SNP_155	Serine hydrolase-like protein	Downstream	C/T		3	CAGCTTTGGGTTGCCTCTAC	CAGGTTGTCACAGGATGTGC	TRINITY_DN12384_c0_g2
SNP_170	Phosphorylase b kinase regulatory subunit alpha	Downstream	C/T		3	GGCCAGGCTGATGTAGAGAG	AGGAATAGGACTGCGACTGC	TRINITY_DN1332_c0_g1
SNP_179	Solute carrier family 22 member 4	Downstream	G/A		1	GAAGGGGGAAAATCTTGAGC	GCTGTAATGCATGGCACATT	TRINITY_DN141_c0_g1
SNP_185	RNA-binding protein 3	Downstream	A/G		3	GGAATTCCCAGGGACAAGG	GGACCCCAATTAACAACAGG	TRINITY_DN1462_c0_g1
SNP_32	Prohibitin-2	Downstream	T/C		5	CATTGCTGACCAGCAGAAGA	CAAATTTAACCGGTGGGATG	TRINITY_DN5661_c0_g1
***SNP_28**	Ferritin heavy chain A	Missense	G/A	Gly/Ser	1	AGACCCCCACCTCTGTGACT	AGGCGGTCGAACAGGTACTC	TRINITY_DN16920_c0_g1

SNP_29	Pleckstrin homology domain-containing family M member 3	Missense	A/G	Asn/Ser	2	CTTTGGACTGGGGACAGAAA	TCATGGATAGGAAGAGCTCCA	TRINITY_DN4049_c0_g1
SNP_111	Smoothelin-like protein 1	Upstream	A/T		1	AGGATGTCAAACGTGGCTCT	GGCTGAGGATGGGTTGAAG	TRINITY_DN1079_c0_g1
SNP_151	Cilia- and flagella-associated protein 20	Upstream	C/A		1	TGGTGTGTCAGGTCTTCTGG	CTCTACTCCCACCCCTTCGT	TRINITY_DN12141_c0_g2
Gene Function: Cellular Housekeeping (15 SNPs)								
SNP_124	Protein CLEC16A	Downstream	C/T		4	ACACAGCATCCGAACATTCA	GCACTCCCAGAGGACAAAAA	TRINITY_DN1101_c0_g2
SNP_149	Sugar phosphate exchanger 3	Downstream	G/C		3	TCTCTGCACAGATGGACCTC	TGCACACTATTTGTCTGCTTCA	TRINITY_DN12094_c0_g1
SNP_165	Tyrosyl-DNA phosphodiesterase 2	Downstream	C/T		3	GGTTAACCAGCATGACAGCAT	CGGACGATGTTACAGGGACT	TRINITY_DN12921_c0_g1
***SNP_176**	PiggyBac transposable element-derived protein 5	Downstream	C/T		4	TCTATTCTCTATCCCCAATCCTTC	GAAACATCTTTGGGGGAAAA	TRINITY_DN1395_c1_g1
SNP_181	Endonuclease III-like protein 1	Downstream	C/T		3	CAACAACGGTGCTGTTTGTC	CTGGATGAGCCCTCAGAGTC	TRINITY_DN14321_c0_g1
SNP_191	Carbohydrate deacetylase	Downstream	T/C		4	GCCACAACATCTGCTCAAAA	GCCATCAGGTCTGAAAGGAG	TRINITY_DN14994_c0_g1
***SNP_1**	Adenomatous polyposis coli protein	Missense	G/A	Met/Ile	3	CAAAGTCCCTCCCAGAATGA	TGCTGTCCAAAAGGTGTCTG	TRINITY_DN47193_c0_g1
SNP_10	Serine/threonine-protein kinase LATS2	Missense	C/T	Ser/Asn	4	ATACGTGTTGCCGTGGAGAT	GGTCCAGCCTTTGCTAATGA	TRINITY_DN374206_c0_g1
SNP_12	Calcium/calmodulin-dependent protein kinase kinase 1	Missense	G/A	Ala/Thr	3	TGCTCACTTGAGGCATGTTC	CGATCGTTGGTCTCCTCATT	TRINITY_DN2565_c0_g2
SNP_13	DNA repair protein complementing XP-C cells	Missense	G/A	Arg/Lys	2	TCCAGCCTTATCTCAAGCAAA	ACATTGCCGTGAACACAGTC	TRINITY_DN4921_c0_g1
SNP_21	CST complex subunit CTC1	Missense	C/G	Ser/Cys	4	TGGTGCCAGACAGAGAAGAA	AAAGCAACTGGGATGGACTG	TRINITY_DN252_c0_g1
SNP_77	Nuclear receptor-interacting protein 1	Missense	C/G	Ala/Gly	4	CAGCAGAAGTGATGCTGAATTT	CCCTTTTTCTTGCCCTCTGT	TRINITY_DN3652_c2_g1

SNP_128	TBC1 domain family member 5	Synonymous	A/T	Pro/Pro	4	GCAGAAGCTCCATCTCGTTC	TTCCCAAACTCTCCCAACCT	TRINITY_DN11179_c0_g1
SNP_162	Ribosomal RNA processing protein 1 homolog B	Synonymous	G/C	Val/Val	1	GAATCTGCACCCTCCAGAAA	CCATTGGCTTCGACAGAGTT	TRINITY_DN1284_c0_g1
SNP_100	Corticotropin-releasing factor receptor 1	Upstream	G/A		1	GACCCTGTTCCTCCAGATCA	GTTCCCTAGACTGGCTTCCA	TRINITY_DN10168_c0_g1
Gene Function: Immune Response (18 SNPs)								
SNP_97	Dynein light chain Tctex-type 1	Downstream	A/T		1	CAAGGTGCTAATACTGAAATCTGC	TGCTAACCAAAAGTACAAAGTGTGA	TRINITY_DN10011_c1_g1
SNP_98	Neural cell adhesion molecule 1	Downstream	A/G		1	TTCGTTGTGCACTTGCTTTT	TTGAGCTTCCCAACCACATT	TRINITY_DN1002_c3_g1
SNP_104	Guanine nucleotide exchange factor VAV3	Downstream	A/G		4	TTGATGGAGTAATTGACAGCATTT	CAGGCAAAACTGGGAAAGAA	TRINITY_DN10542_c0_g1
SNP_138	RING-type E3 ubiquitin-protein ligase PPIL2	Downstream	C/G		4	AGAGAAATGCCCTCCCTACC	TTTTTCAAGCACTCAAAAGAAAA	TRINITY_DN115310_c0_g2
SNP_139	Protein C12orf4 homolog	Downstream	C/T		3	GCTGCTGCTGACTCCTGATT	GCAACTGTTCCCAGTGTCCT	TRINITY_DN1159_c0_g1
SNP_147	Group XV phospholipase A2	Downstream	A/T		2	TGTGGTAAAATGAAGCTGAAGG	CAAGCGATCAAAGAACACCA	TRINITY_DN12040_c0_g1
SNP_157	Gamma-interferon-inducible lysosomal thiol reductase	Downstream	C/T		2	GGCAACATGATGGAGGTGAC	ACTCCAGGCAGAAGATGACG	TRINITY_DN12517_c0_g1
SNP_172	Endogenous retrovirus group K member 6 Pol protein	Downstream	C/T		4	TTGTTACTGTGGGTGCAGTTT	TTGTCTTTGATCACGCTTGC	TRINITY_DN1345_c0_g1
SNP_173	Ubiquitin-like protein ATG12	Downstream	A/G		3	TTTTGACACTTTCCCCTTGG	TTGACATGTTTGCAGTATGGTTT	TRINITY_DN1364_c0_g2
SNP_188	Lysosome-associated membrane glycoprotein 2	Downstream	A/G		3	TCTGGAAGTGGCAGCAGTAA	GCATCTGGAAACAGCACTGA	TRINITY_DN14740_c0_g3
***SNP_23**	Golgin subfamily B member 1	Missense	C/A	His/Asn	3	CCAGAAAGCTCACCGAACATA	TTGGCAACACATCTCTTTGG	TRINITY_DN2009_c0_g1

SNP_78	Vezatin	Missense	G/C	Gly/Ala	3	ATTTGGGATGTCTCG CTGTT	GTGTTTTCGCAGG GACATTT	TRINITY_DN 50902_c0_g2
***SNP_88**	NACHT	Missense	A/G	Thr/Ala	3	CTGCACCTCCAGGAG TTTTT	GCTGACCAAACCA AAGAGGA	TRINITY_DN 3426_c0_g1
SNP_89	Zinc finger protein 40	Missense	T/C	Val/Ala	2	AGCAGCAAGGCCAGT ACTTC	TGGCTGTTCACTGG AGACAA	TRINITY_DN 6008_c1_g1
***SNP_133**	Coiled-coil domain-containing protein 130	Synonymous	G/C	Arg/Arg	4	ATGTTCCGCCTGGAG CAC	GTGCTTTTAGGGC GTTTTGG	TRINITY_DN 11342_c0_g1
SNP_99	Paired amphipathic helix protein Sin3a	Synonymous	G/A	Glu/Glu	1	AGGTGAACTCTCGGA TGTGG	AGCTTGGTTTTGGG AGGACT	TRINITY_DN 10063_c0_g1
SNP_137	Enhancer of filamentation 1	Upstream	T/C,G		4	CCTGACGGTGATAGA GCAGA	GATCTTTTGGTGGC TGAAGG	TRINITY_DN 11498_c0_g1
***SNP_144**	Bifunctional heparan sulfate N-deacetylase/N-sulfotransferase 1	Upstream	T/C		5	GCCATCACCACAGGA GTTTT	GAAGAAGCAAGCA ACCAACC	TRINITY_DN 118147_c1_g1
Gene Function: Lipid Metabolism (16 SNPs)								
SNP_113	Pleckstrin homology domain-containing family A member 3	Downstream	T/C		1	CACCAGAAGATGGAT TCTGC	TTGACCAAGGTGC TAGTAGGC	TRINITY_DN 1086_c0_g1
SNP_114	Group XIIA secretory phospholipase A2	Downstream	G/A		3	AAACACCCCAAGCCA ATGTA	TCACGGTTCTGTCA AATCAAA	TRINITY_DN 10324_c1_g1
SNP_119	Extended synaptotagmin-2	Downstream	A/G		5	GTGGAAGGATTTTTG CTCCA	CACATTTGCCTGA ACACAGC	TRINITY_DN 1094_c0_g1
SNP_136	Beta-1	Downstream	A/G		2	TCGTCTCCACATTCCT CCTC	ATCGTCTGATCTCC CACCAG	TRINITY_DN 11464_c0_g1
SNP_148	Actin-related protein 5	Downstream	A/C		1	GTGGAGGGGAAAACT CCTTC	AGCCCAGCTGCAA TAAAAAC	TRINITY_DN 12054_c0_g1
SNP_150	Lanosterol 14-alpha demethylase	Downstream	G/A		1	TGGAAAATTTTGTTG GCATTC	GGGATGGTTCTTCC AAACAT	TRINITY_DN 1209_c0_g1
SNP_152	Elongation of very long chain fatty acids protein 1	Downstream	T/C		4	ACTTTCCCCAAGTGC CTACA	CTCACACATGAGC TGGCAGT	TRINITY_DN 12322_c0_g2
SNP_156	Hexosaminidase D	Downstream	A/G		2	TGTGCTTCGTTTATGC CTTTC	TCTGCATGTGGAC CTGGTTA	TRINITY_DN 12444_c0_g1

SNP_168	Long-chain-fatty-acid--CoA ligase 3	Downstream	T/A		5	CAAGGCTGTAGGGAAGTGTCA	AAATCTGCTGCACAATGCAC	TRINITY_DN1317_c0_g1
SNP_187	GPI mannosyltransferase 2	Downstream	T/G		3	AGCTGCTCTGAAAGCCTGAG	GGCAGGTGATGGGAATTTT	TRINITY_DN14733_c0_g1
SNP_42	Patatin-like phospholipase domain-containing protein 2	Downstream	C/T		3	TCGTTCTTCATACTGCCACCT	CATCAGGATTTGGGAGGAAA	TRINITY_DN4684_c0_g1
SNP_35	ATPase MORC2	Missense	C/T	Pro/Ser	2	AGCTAGCCAGCATGCTCTTC	ACGTAAGCTTTTGGGGCTCT	TRINITY_DN1611_c0_g1
SNP_37	Palmitoyl-protein thioesterase 1	Missense	A/G	Glu/Gly	2	ACAGCACTGCAGGGATCTG	TCTGAGAACAGGGGTGGATT	TRINITY_DN4241_c0_g2
SNP_41	Activin receptor type-2A	Missense	A/G	Ser/Pro	2	CACGATCAGAAGGCAGTGG	GACGCGTTCCTGAGGATAGA	TRINITY_DN5907_c1_g2
SNP_161	Lathosterol oxidase	Synonymous	C/T	Arg/Arg	2	ACGTCTGGACCATCTCCATC	AAGAACCCCCAATCTTGTCC	TRINITY_DN1283_c0_g1
***SNP_127**	Monoglyceride lipase	Upstream	G/C		5	CAAGCGACTTTCCTCCAAGA	GTCAAACGCAAGCAGATGAG	TRINITY_DN1113_c1_g1
Gene Function: Nervous System Development (19 SNPs)								
SNP_105	Ankyrin repeat and LEM domain-containing protein 2	Downstream	G/A		1	CAGGAAGTCCAGGGAAACAC	CTGAAGTCCCAGGATGAGGA	TRINITY_DN10547_c1_g1
SNP_154	Tomoregulin-2	Downstream	A/T		1	ACCTGGCTGGAAGACAAGTG	CTGCCTCATTTGGTAGTTGC	TRINITY_DN12341_c0_g1
SNP_159	Potassium voltage-gated channel subfamily A member 2	Downstream	C/A,G		1	CAGCTGGGGAAGGTCAGG	CAGCATGCAGCATTTTCAGT	TRINITY_DN1265_c0_g1
SNP_160	Tenascin-R	Downstream	T/C		4	ACACCATCCCTCTTCAATGC	ATCCAAAGGGTCCATCTTCC	TRINITY_DN126_c0_g1
SNP_182	Neuromodulin	Downstream	G/A		1	CTATGCTGCCGTACATCCTG	GCTCCCTTAAAATCCCCTCA	TRINITY_DN14461_c0_g2
SNP_49	Synaptojanin-1	Missense	C/A	Arg/Ser	3	GTGCCGCTGACTGCTTCT	TGGAACAAACACAACCTTGC	TRINITY_DN4744_c0_g1
SNP_50	Peripheral-type benzodiazepine	Missense	C/G	Ser/Cys	3	ACCAGTGTCGCAGTCAAACA	GCTGAGTCCTTCTCCCAGTG	TRINITY_DN492_c0_g1

	receptor-associated protein 1							
SNP_52	Neurabin-1	Missense	G/A	Gly/Asp	2	TGCAACAGAAGGAACAGTCG	TTCACATCTTCATGCCCATC	TRINITY_DN2242_c1_g1
***SNP_53**	Zinc finger protein 106	Missense	T/A	Ser/Thr	2	TTCAAAGCATTCGGTCCTTC	TGGGCTGCTCTCAGAGTTTT	TRINITY_DN3442_c0_g1
SNP_54	Disabled homolog 1	Missense	A/G	Thr/Ala	1	AGTCGCTCAGGTGATGCAG	GGCTGCTTGTAAAGGCAAAA	TRINITY_DN663_c0_g1
SNP_55	Protein phosphatase Slingshot homolog 1	Missense	A/G	Thr/Ala	5	GCCTTTTGGAGAGAGGGAAA	CAGGTCCTTGGTAGGTCTGG	TRINITY_DN8609_c0_g1
SNP_56	Activated CDC42 kinase 1	Missense	G/A	Val/Met	4	AAGGTCAGCAGCACCCACTA	GAAAAGTTGCCCTTGCAGTC	TRINITY_DN1426_c0_g1
SNP_61	Rho GTPase-activating protein 35	Missense	G/A	Gly/Ser	5	CGCCAAGGACAAGTACGAG	GATGTGCTCCTGCTTGAGG	TRINITY_DN11794_c0_g1
***SNP_62**	Methyl-CpG-binding domain protein 5	Missense	G/A	Ala/Thr	5	CAGCAGGCCAAGGACACC	GAGGCTGTGAAGGCACTCAT	TRINITY_DN8045_c0_g1
SNP_63	Dickkopf-related protein 3	Missense	A/G	Thr/Ala	2	CTGCCAATGAAACACAGCAC	TGGTCCTCCAGGCTTTCTAA	TRINITY_DN8355_c0_g1
SNP_64	Microtubule-associated protein 1A	Missense	G/C	Ala/Pro	2	GCAGCATGAACAGGTTTTGA	TTTCTGGTTTTGTGCTTGGA	TRINITY_DN6199_c0_g2
SNP_175	Protocadherin gamma-C5	Synonymous	G/A	Pro/Pro	3	CTTCTCCCTGGATGTCAAGC	CAGCACCTGCACCGTTATC	TRINITY_DN1393_c0_g2
SNP_178	Protein shisa-9	Synonymous	C/T	Leu/Leu	1	ATGGGCAAGATTCACACACA	GTGGTCAGCGGGTCACTTAG	TRINITY_DN141456_c0_g2
SNP_112	Fibroblast growth factor 14	Upstream	C/T		1	CCAGGAACAACAACCCTTTG	AGTGGCATCTCTGTGGCATT	TRINITY_DN1085_c0_g4
Gene Function: Reproduction (18 SNPs)								
SNP_116	Lysine-specific demethylase 5A	Downstream	A/G		2	ACCTGGGAAGAGGGAAGTGT	CCACAGCTTGCTTTTGCTTT	TRINITY_DN10919_c0_g1
SNP_145	Spermatogenesis-associated protein 20	Downstream	G/T		5	AAAGCACCTGGATGACTTGG	GGGAGGACAAGGAGGAAAGA	TRINITY_DN1190_c1_g1
SNP_158	Cytidine and dCMP deaminase domain-containing protein 1	Downstream	G/A		3	CTTCCTGCAGTCTTGCTTCA	TTTCGCCAGGAGCTTCTAAA	TRINITY_DN12544_c0_g1

SNP_94	Neuronal PAS domain-containing protein 2	Downstream	C/T		2	GCTTTGTTGTGTTGGTGGTG	AGCTTGAAAATGGAGCTTGG	TRINITY_DN43689_c2_g1
SNP_95	Ankycorbin	Downstream	A/G		2	CTTGGGTACCATGGCTTCAT	ACCGTCAGGTAATCAATGCAC	TRINITY_DN101614_c3_g1
SNP_96	Endophilin-A3	Downstream	A/G		4	GGCAGTTTCTTTGCTGGAGT	AGCCAACTGGCTGACTTGTT	TRINITY_DN10448_c0_g1
SNP_24	BTB/POZ domain-containing protein KCTD17	Missense	T/A	Cys/Ser	2	AAGGAGGGAGGTGTGAGGTT	TGCTTTGCATTCATTTCCAC	TRINITY_DN365_c0_g1
SNP_59	Plexin-A2	Missense	G/C	Glu/Gln	2	GACCCCAAGTTCCACTCGTA	AAGATGGCAAAGAGCACGTC	TRINITY_DN55_c0_g1
SNP_60	Receptor-type tyrosine-protein phosphatase zeta	Missense	A/C	His/Pro	4	GTTCTTTCCCAAGGCTCCAT	GCATCAGCGTAACTGGTCTG	TRINITY_DN2349_c0_g1
SNP_74	Tubulin polyglutamylase TTLL5	Missense	C/G	Pro/Ala	2	CACAGTCCAGCACCAGTCAT	TTTGTTGGAGGCTTTGGAAC	TRINITY_DN1987_c2_g1
SNP_75	Hyaluronidase-3	Missense	A/G	His/Arg	3	ACTACGGCATCGTGGAGAAC	GGCCACCCTGTTGATGTG	TRINITY_DN2610_c0_g1
***SNP_76**	Testis-expressed protein 30	Missense	A/G	Ile/Val	5	GGGCGGAGGTTAAAGTGAA	AAGATAGGCTGCCAAGGACA	TRINITY_DN5351_c0_g1
SNP_79	Group 3 secretory phospholipase A2	Missense	G/A	Glu/Lys	5	CCATCATCCAACACCATCCT	AGGCTCATGGAGGACTCAGA	TRINITY_DN6807_c0_g2
***SNP_80**	Regulator of nonsense transcripts 1	Missense	G/A	Ala/Thr	1	GAAGAACCGCTTTGGGATTC	CCAGGCTGACTCATCTGTGA	TRINITY_DN17794_c0_g1
SNP_81	Fanconi anemia group M protein	Missense	T/G	Ser/Arg	2	GCCACCTTTAAAGCAACCAA	CTCCATCCCCTCGTCCTT	TRINITY_DN1800_c0_g1
SNP_146	Sterile alpha and TIR motif-containing protein 1	Synonymous	T/C	Cys/Cys	4	CCTTCTCCAAAGACGACGAG	CCTGGATGTTGTCACTGCTG	TRINITY_DN11971_c0_g3
SNP_135	Katanin p60 ATPase-containing subunit A-like 1	Upstream	C/T		4	GCTGGGTTGTGGTCTGATG	TGACTTGACTCTGCGACTGG	TRINITY_DN11461_c0_g1
SNP_171	Iron-sulfur cluster assembly 1 homolog	Upstream	C/T		4	TTGCCAAACAAAAACATGGA	ATGCTCTCCACCCCAAAAC	TRINITY_DN133303_c0_g4

Gene Function: Stress Response (21 SNPs)								
SNP_117	Thioredoxin-interacting protein	Downstream	T/C		1	AAAAATGCCACGTTCCTGAG	GAGATTTGAGACGGGGAACA	TRINITY_DN10923_c0_g1
SNP_122	Glutamate--cysteine ligase regulatory subunit	Downstream	G/C		4	CACCCCATGTCTTCGTTCTT	CACCCCACAGAAATTCTTCC	TRINITY_DN1099_c0_g1
***SNP_130**	Serum paraoxonase/arylesterase 2	Downstream	G/A		2	CCCTTGACCATTTCAACAGC	ATGAGCAGCTTTCCCTGGTA	TRINITY_DN11266_c0_g1
SNP_131	Ribonuclease inhibitor	Downstream	T/C		1	TCTGGGGAAGGCTTACAAAA	TTTGACATTGCACAGCTGAA	TRINITY_DN11267_c0_g1
SNP_140	Transcription regulator protein BACH2	Downstream	T/C		5	TGGGTCTGGTGAAGTCAGTG	ATGCTGCAGGATGAGAGGAT	TRINITY_DN116331_c0_g4
SNP_174	Apoptosis regulator Bcl-2	Downstream	G/A		1	GCAAATGCATAGGCATCAAA	ACAGATCTCAGGTGATCCTACAGA	TRINITY_DN13824_c0_g2
SNP_180	TAR DNA-binding protein 43	Downstream	C/A		3	TGAGGGTTTTTCTGTTGTGTG	CCTGCTCTCAGCTGCTACCT	TRINITY_DN141_c0_g2
SNP_190	Phosducin-like protein 3	Downstream	T/G		1	CTCTTACCCCACTGTGCTCTG	CTTACAGCCTCCTGCTGTCC	TRINITY_DN14955_c0_g1
SNP_192	Transducin beta-like protein 2	Downstream	G/A		4	TAAAGGCTACCCCTGCAGAA	AGAGATCCCGCAAGAGACAA	TRINITY_DN14_c0_g2
***SNP_71**	Serum paraoxonase/arylesterase 2	Downstream	G/A		2	CCCTTGACCATTTCAACAGC	ATGAGCAGCTTTCCCTGGTA	TRINITY_DN11266_c0_g1
SNP_72	DnaJ homolog subfamily C member 3	Downstream	T/G		4	TGTTGAAGCAGAACCCTTGG	GCTGCTGTGGTGGTTTTGTA	TRINITY_DN5378_c0_g1
***SNP_14**	UbiA prenyltransferase domain-containing protein 1	Missense	C/G	Arg/Pro	5	TAATTTGTCCACCGGGAGAT	AGCACCTGGAAGGGGAAG	TRINITY_DN4509_c0_g1
***SNP_17**	Glutathione peroxidase 1	Missense	C/G	Arg/Ser	5	TCCCTGTTAGCTGAGGGTTT	CCACATTGACCACCAGCA	TRINITY_DN3586_c2_g1
***SNP_19**	Coiled-coil-helix-coiled-coil-helix domain-containing protein 2	Missense	G/C	Asp/Glu	5	GGTTTGCGATCACCATGA	GCAGCAGTTGAGGCCATCT	TRINITY_DN36305_c0_g1

SNP_7	SNF-related serine/threonine-protein kinase	Missense	C/A,G	Asp/Glu	5	CCTGCCGTTGACACCACTA	CAGGAAGGCTCGCATCTG	TRINITY_DN7026_c0_g2
SNP_108	Neuroepithelial cell-transforming gene 1 protein	Synonymous	G/A	Glu/Glu	1	CCCATGCTGAAACTCTCCAT	GCCCAATCTGTTCCACTGTT	TRINITY_DN10664_c0_g1
SNP_121	Voltage-dependent T-type calcium channel subunit alpha-1H	Synonymous	G/A	Glu/Glu	3	AGGACTCGCAGAACCTGCT	ATGGATCCTCTTTGGGCTTT	TRINITY_DN10972_c0_g1
SNP_70	Heat shock cognate 71 kDa protein	Synonymous	C/G	Ser/Ser	4	ACGAGGGCATCGACTTCTAC	CTGCAGCAGCTTCTGGATCT	TRINITY_DN9898_c0_g1
SNP_118	Solute carrier family 23 member 2	Upstream	C/G		4	GCTGCTGGAATAAGGAGCTG	GTGCTTGGACTCATCCTCGT	TRINITY_DN10925_c1_g1
SNP_132	Renin receptor	Upstream	T/C		3	TTGTGTTCGCTCAGAACAGG	TGGCAGAAAAGTCACTCCAG	TRINITY_DN11329_c0_g1
SNP_68	Thioredoxin-related transmembrane protein 4	Upstream	T/G		2	GTCATGTGCAGTGCAGTCCT	ACCTGTGCCCCCTCTATTTC	TRINITY_DN15772_c0_g1

Appendix A4: Justification for our choice of genes for SNP loci development for population genetic analyses of six breeding snow buntings (*Plectrophenax nivalis*) populations.

Broad functional category	Justification	Examples of specific gene functions
Energetics	Snow buntings have thermogenic capacity and cold acclimatization (Le Pogam et al., 2021) to breed in harsh Arctic conditions. Different local climates may be drive variation in metabolic performance across populations.	- Muscle contraction - Muscle hypertrophy - Vascularization - Mitochondrial assembly - Erythrocyte production and destruction
Lipid metabolism	Patterns of lipid storage and utilizations for energy production may be habitat-dependent due to selective pressures from the abiotic factors (e.g., temperature, food availability) or biotic factors (e.g., interspecific competition for resources).	- Lipid synthesis - Lipid degradation
Immune response	Immune response variation helps individuals fight off various pathogens and viruses that may be site-specific.	- Immunoglobulin protein structures - Antigen processing - T cell activation - Autophagy - Viral response
Stress response	Individuals can vary in stress response due to local conditions as they are experience site-specific temperatures, resource availability, contaminants and predators.	- Heat shock protein - Genotoxic stress control - Cell redox homeostasis - Hypoxia stress
Nervous system development	Neuronal health and development differences in individuals may control decisions relating to various aspects of survival and reproduction.	- Neural tube development - Vertebrate development - Neurite formation - Musculoskeletal movement control - Neurotransmitter transport
Reproduction	Individual reproductive biology, phenology and behaviours may be habitat dependent due to	- Cilium and flagellum movement - Spermatogenesis and sperm polarity

	differential breeding density, breeding synchrony and food availability.	- Testicular development - Embryonic viability - Circadian rhythm - Migration
Cellular housekeeping	Individuals should be genetically similar at vital-function genes involved in regular cellular housekeeping regardless of habitat-based differences. These were included as control genes since they are expected to be highly canalized across populations.	- Activation of signal transduction pathways - DNA repair - Apoptosis - Regulation of certain pathways (i.e. Fanconi anemia) to prevent disease - Telomerase maintenance

Appendix A5: Primer sequence, repeat motif and amplicon size of the nine microsatellite loci used for assessing reproductive isolation and neutral genetic divergence. The species used to develop the original primer sets is given.

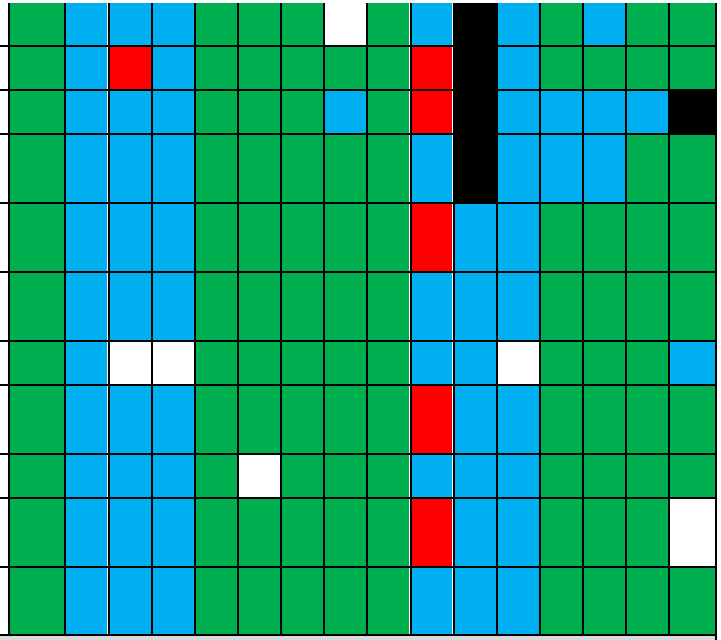
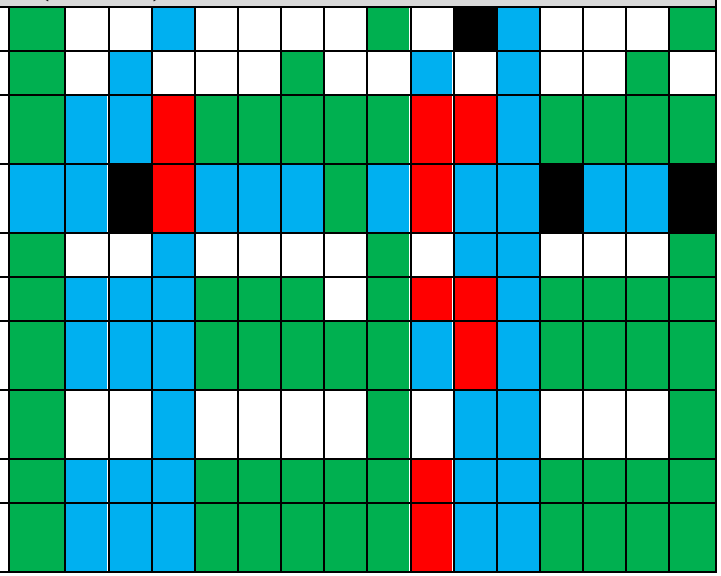
Primer	Origin	Primer Sequence (5' to 3')	Core Repeat	Size Range	Reference
SNBU 682	*P. nivalis* (snow bunting)	**F:** ACCTGCTGTTGTTGAGGAGA **R:** AGGAAGACAAGTAATAATGAATGCAGT	ACAG	208-237	This study
SNBU 705	*P. nivalis* (snow bunting)	**F:** AACAGCCTCCTCCTTGGATG **R:** TGTATAAACTCTTGTGCATGTTCTG	ATCC	160-302	This study
Gf12	*Geospiza fortis* (ground finch)	**F:** TTTGGGTTTGCCTCCCTA **R:** CAGTGCAGCAACATGGTTT	AC	98-131	Petren, 1998, F' & R' modified in this study
INDIGO29	*Vidya chalybeate* (village indigobird)	**F:** CCAGAACTGAGCCTAGGAAA **R:** GGAAGAAGGCTGGGTAAAAT	CA	136-171	Sefc et al., 2001, F' modified in this study
LOX8	*Loxia scotica* (Scottish crossbill)	**F:** GATTTAAAATGCTTAGTATGAAGCA **R:** AGTTGAGGCCATTAAAAAGATTC	CTTT, CCTT	184-251	Piertney et al., 1998, F' modified in this study
Cuu28	*Catharus ustulatus* (Swainson's thrush)	**F:** GAGGCACAGAAATGTGAATT **R:** TAAGTAGAAGGACTTGATGGCT	CA	175-198	Gibbs et al., 1999
CAM 17	*Taeniopygia guttata* (zebra finch) & *Gallus gallus* (chicken)	**F:** CGGGTTGTAATCAAGAAGATGC **R:** CTGCGGAGCAATTAACGC	N/A	221-227	Dawson et al., 2010
Ecit 2	*Emberiza citronella* (yellowhammer)	**F:** TTCAGCCAAGACAGATAAAAA **R:** CACTTTCAGATGCCATTTCAG	GT	155-170	Wonke et al., 2007
POCC6	*Phylloscopus occipitalis* (western crowned warbler)	**F:** TCACCCTCAAAAACACACACA **R:** ACTTCTCTCTGAAAAGGGGAGC	CA	197-207	Bensch et al., 1997

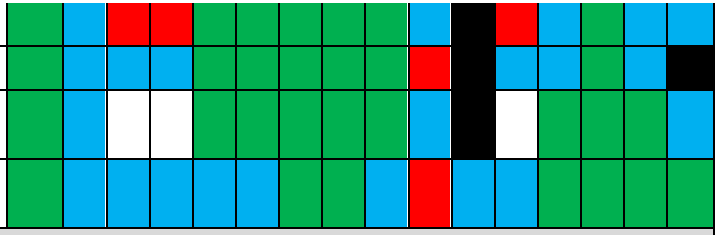
Appendix A6: Sample sizes (N) and observed heterozygosity values (H_{obs}) for six breeding snow bunting (*Plectrophenax nivalis*) populations at microsatellite and Single Nucleotide Polymorphism (SNP) loci. See Table 2.2 for description of sampled population codes.

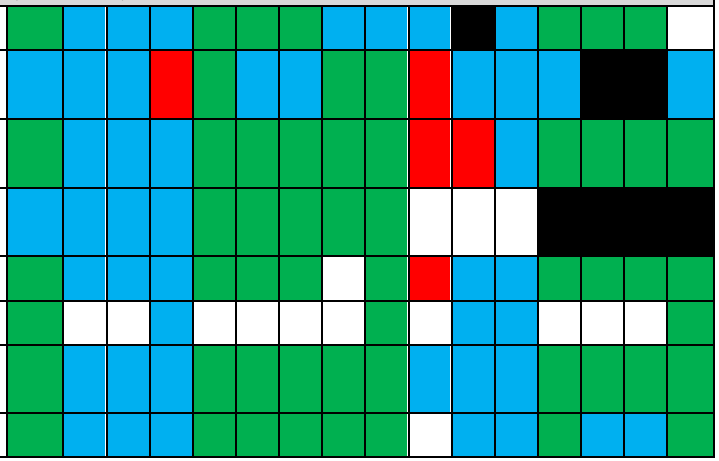
Population	**Microsatellite Loci**		**SNP Loci**	
	N	**H_{obs}**	**N**	**H_{obs}**
A	51	0.635	51	0.1
S	33	0.63	33	0.101
B	51	0.635	50	0.107
M	53	0.708	53	0.104
AI	20	0.406	19	0.098
PI	16	0.345	16	0.111

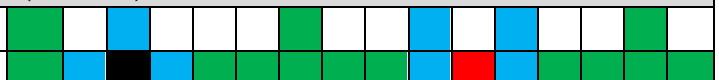
Appendix A7: SNP loci (n=101) results summary for global and pairwise comparisons from population genetic analyses of six snow bunting (*Plectrophenax nivalis*) populations. All loci belong to one of the following categories: Energetics, Cellular Housekeeping, Immune Response, Lipid Metabolism, Nervous System Development, Reproduction, and Stress Response. All results are based on Hedrick's G'_{ST} values determining whether a SNP loci is under divergent selection (black), stabilizing selection (green), genetic drift (blue) or undetermined (red) based on 99% and 99.9% neutral marker ranges for global and pairwise comparisons, respectively. For some loci (white), we were unable to calculate pairwise Hedrick's G'_{ST} value likely due to insufficient sequence reads. See Table 2.2 for description of sampled population codes.

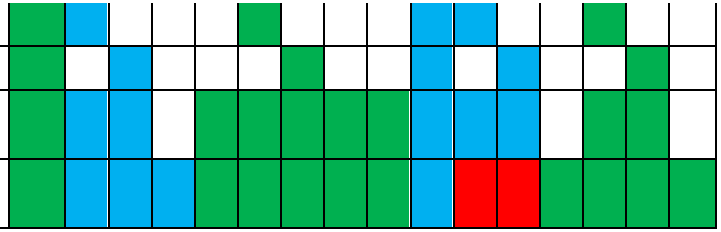
Primer Name	Gene Description	Type of Variant	Nucleotide Variant	Amino Acid Variant	Global Comparison	Pairwise Comparisons														
						A/B	A/M	A/S	AI/A	AI/B	AI/M	AI/PI	AI/S	B/M	B/S	M/S	PI/A	PI/B	PI/M	PI/S
Gene Function: Energetics (9 SNPs)																				
SNP_125	Sodium channel subunit beta-2	Downstream	G/A																	
SNP_155	Serine hydrolase-like protein	Downstream	C/T																	
SNP_170	Phosphorylase b kinase regulatory subunit alpha	Downstream	C/T																	
SNP_179	Solute carrier family 22 member 4	Downstream	G/A																	
SNP_185	RNA-binding protein 3	Downstream	A/G																	
SNP_32	Prohibitin-2	Downstream	T/C																	
SNP_29	Pleckstrin homology domain-containing family M member 3	Missense	A/G	Asn/Ser																
SNP_111	Smoothelin-like protein 1	Upstream	A/T																	
SNP_151	Cilia- and flagella-associated protein 20	Upstream	C/A																	
Gene Function: Cellular Housekeeping (13 SNPs)																				
SNP_124	Protein CLEC16A	Downstream	C/T																	
SNP_149	Sugar phosphate exchanger 3	Downstream	G/C																	

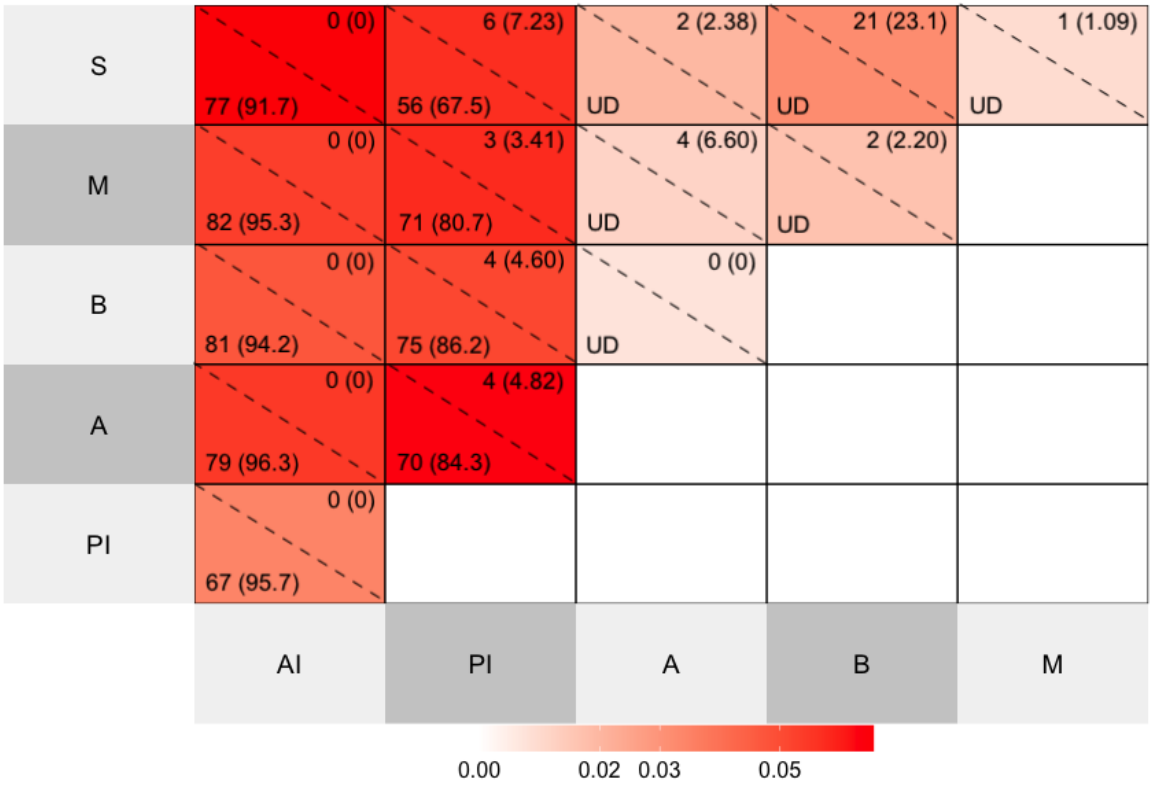
SNP_165	Tyrosyl-DNA phosphodiesterase 2	Downstream	C/T		
SNP_181	Endonuclease III-like protein 1	Downstream	C/T		
SNP_191	Carbohydrate deacetylase	Downstream	T/C		
SNP_10	Serine/threonine-protein kinase LATS2	Missense	C/T	Ser/Asn	
SNP_12	Calcium/calmodulin-dependent protein kinase kinase 1	Missense	G/A	Ala/Thr	
SNP_13	DNA repair protein complementing XP-C cells	Missense	G/A	Arg/Lys	
SNP_21	CST complex subunit CTC1	Missense	C/G	Ser/Cys	
SNP_77	Nuclear receptor-interacting protein 1	Missense	C/G	Ala/Gly	
SNP_128	TBC1 domain family member 5	Synonymous	A/T	Pro/Pro	
SNP_162	Ribosomal RNA processing protein 1 homolog B	Synonymous	G/C	Val/Val	
SNP_100	Corticotropin-releasing factor receptor 1	Upstream	G/A		
Gene Function: Immune Response (14 SNPs)					
SNP_97	Dynein light chain Tctex-type 1	Downstream	A/T		
SNP_98	Neural cell adhesion molecule 1	Downstream	A/G		
SNP_104	Guanine nucleotide exchange factor VAV3	Downstream	A/G		
SNP_138	RING-type E3 ubiquitin-protein ligase PPIL2	Downstream	C/G		
SNP_139	Protein C12orf4 homolog	Downstream	C/T		
SNP_147	Group XV phospholipase A2	Downstream	A/T		
SNP_157	Gamma-interferon-inducible lysosomal thiol reductase	Downstream	C/T		
SNP_172	Endogenous retrovirus group K member 6 Pol protein	Downstream	C/T		
SNP_173	Ubiquitin-like protein ATG12	Downstream	A/G		
SNP_188	Lysosome-associated membrane glycoprotein 2	Downstream	A/G		

SNP_78	Vezatin	Missense	G/C	Gly/Ala	
SNP_89	Zinc finger protein 40	Missense	T/C	Val/Ala	
SNP_99	Paired amphipathic helix protein Sin3a	Synonymous	G/A	Glu/Glu	
SNP_137	Enhancer of filamentation 1	Upstream	T/C, G		
Gene Function: Lipid Metabolism (15 SNPs)					
SNP_113	Pleckstrin homology domain-containing family A member 3	Downstream	T/C		
SNP_114	Group XIIA secretory phospholipase A2	Downstream	G/A		
SNP_119	Extended synaptotagmin-2	Downstream	A/G		
SNP_136	Beta-1	Downstream	A/G		
SNP_148	Actin-related protein 5	Downstream	A/C		
SNP_150	Lanosterol 14-alpha demethylase	Downstream	G/A		
SNP_152	Elongation of very long chain fatty acids protein 1	Downstream	T/C		
SNP_156	Hexosaminidase D	Downstream	A/G		
SNP_168	Long-chain-fatty-acid--CoA ligase 3	Downstream	T/A		
SNP_187	GPI mannosyltransferase 2	Downstream	T/G		
SNP_42	Patatin-like phospholipase domain-containing protein 2	Downstream	C/T		
SNP_35	ATPase MORC2	Missense	C/T	Pro/Ser	
SNP_37	Palmitoyl-protein thioesterase 1	Missense	A/G	Glu/Gly	
SNP_41	Activin receptor type-2A	Missense	A/G	Ser/Pro	
SNP_161	Lathosterol oxidase	Synonymous	C/T	Arg/Arg	
Gene Function: Nervous System Development (17 SNPs)					
SNP_105	Ankyrin repeat and LEM domain-containing protein 2	Downstream	G/A		
SNP_154	Tomoregulin-2	Downstream	A/T		

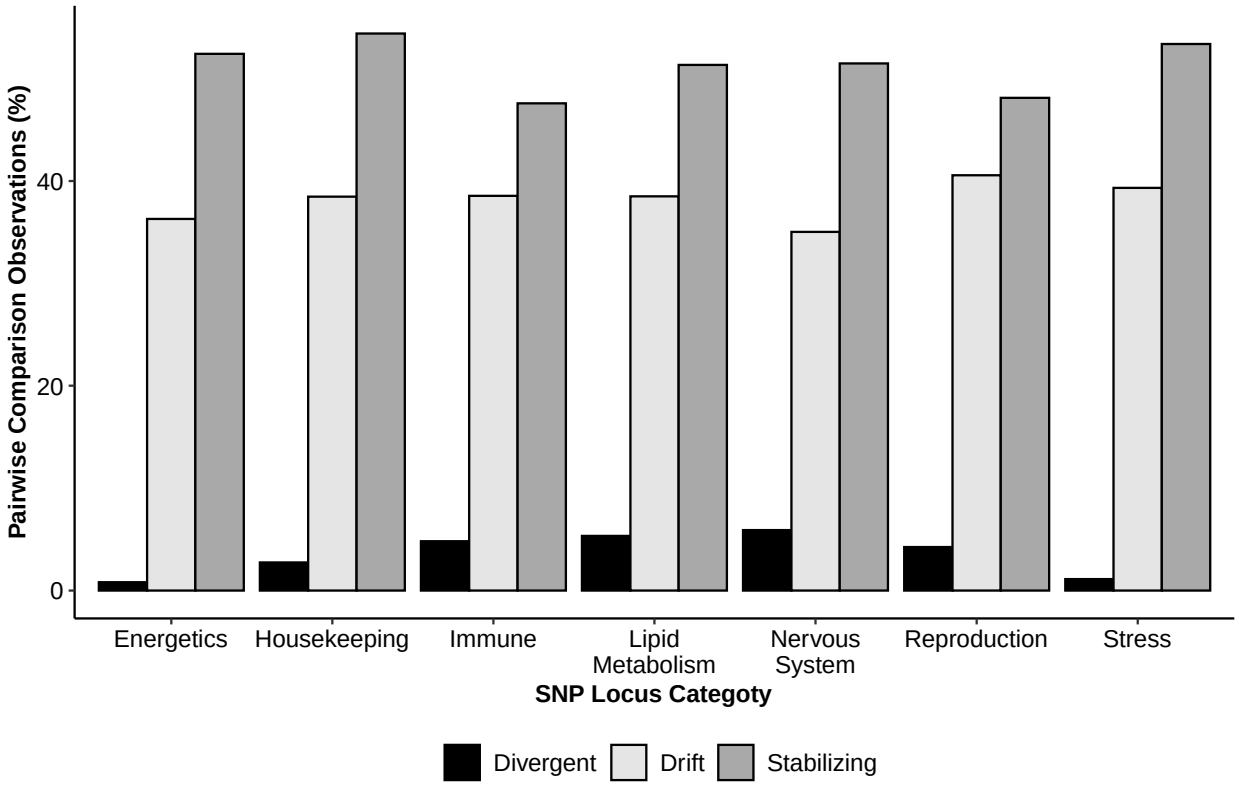
SNP_159	Potassium voltage-gated channel subfamily A member 2	Downstream	C/A, G		
SNP_160	Tenascin-R	Downstream	T/C		
SNP_182	Neuromodulin	Downstream	G/A		
SNP_49	Synaptojanin-1	Missense	C/A	Arg/Ser	
SNP_50	Peripheral-type benzodiazepine receptor-associated protein 1	Missense	C/G	Ser/Cys	
SNP_52	Neurabin-1	Missense	G/A	Gly/Asp	
SNP_54	Disabled homolog 1	Missense	A/G	Thr/Ala	
SNP_55	Protein phosphatase Slingshot homolog 1	Missense	A/G	Thr/Ala	
SNP_56	Activated CDC42 kinase 1	Missense	G/A	Val/Met	
SNP_61	Rho GTPase-activating protein 35	Missense	G/A	Gly/Ser	
SNP_63	Dickkopf-related protein 3	Missense	A/G	Thr/Ala	
SNP_64	Microtubule-associated protein 1A	Missense	G/C	Ala/Pro	
SNP_175	Protocadherin gamma-C5	Synonymous	G/A	Pro/Pro	
SNP_178	Protein shisa-9	Synonymous	C/T	Leu/Leu	
SNP_112	Fibroblast growth factor 14	Upstream	C/T		
Gene Function: Reproduction (16 SNPs)					
SNP_116	Lysine-specific demethylase 5A	Downstream	A/G		
SNP_145	Spermatogenesis-associated protein 20	Downstream	G/T		
SNP_158	Cytidine and dCMP deaminase domain-containing protein 1	Downstream	G/A		
SNP_94	Neuronal PAS domain-containing protein 2	Downstream	C/T		
SNP_95	Ankycorbin	Downstream	A/G		
SNP_96	Endophilin-A3	Downstream	A/G		
SNP_24	BTB/POZ domain-containing protein KCTD17	Missense	T/A	Cys/Ser	
SNP_59	Plexin-A2	Missense	G/C	Glu/Gln	

SNP_60	Receptor-type tyrosine-protein phosphatase zeta	Missense	A/C	His/Pro	
SNP_74	Tubulin polyglutamylase TTLL5	Missense	C/G	Pro/Ala	
SNP_75	Hyaluronidase-3	Missense	A/G	His/Arg	
SNP_79	Group 3 secretory phospholipase A2	Missense	G/A	Glu/Lys	
SNP_81	Fanconi anemia group M protein	Missense	T/G	Ser/Arg	
SNP_146	Sterile alpha and TIR motif-containing protein 1	Synonymous	T/C	Cys/Cys	
SNP_135	Katanin p60 ATPase-containing subunit A-like 1	Upstream	C/T		
SNP_171	Iron-sulfur cluster assembly 1 homolog	Upstream	C/T		
Gene Function: Stress Response (17 SNPs)					
SNP_117	Thioredoxin-interacting protein	Downstream	T/C		
SNP_122	Glutamate--cysteine ligase regulatory subunit	Downstream	G/C		
SNP_131	Ribonuclease inhibitor	Downstream	T/C		
SNP_140	Transcription regulator protein BACH2	Downstream	T/C		
SNP_174	Apoptosis regulator Bcl-2	Downstream	G/A		
SNP_180	TAR DNA-binding protein 43	Downstream	C/A		
SNP_190	Phosducin-like protein 3	Downstream	T/G		
SNP_192	Transducin beta-like protein 2	Downstream	G/A		
SNP_72	DnaJ homolog subfamily C member 3	Downstream	T/G		
SNP_7	SNF-related serine/threonine-protein kinase	Missense	C/A, G	Asp/Glu	
SNP_108	Neuroepithelial cell-transforming gene 1 protein	Synonymous	G/A	Glu/Glu	
SNP_121	Voltage-dependent T-type calcium channel subunit alpha-1H	Synonymous	G/A	Glu/Glu	
SNP_70	Heat shock cognate 71 kDa protein	Synonymous	C/G	Ser/Ser	

SNP_118	Solute carrier family 23 member 2	Upstream	C/G	
SNP_132	Renin receptor	Upstream	T/C	
SNP_144	Bifunctional heparan sulfate N-deacetylase/N-sulfotransferase 1	Upstream	T/C	
SNP_68	Thioredoxin-related transmembrane protein 4	Upstream	T/G	



Appendix A8: Heatmap of pairwise Hedrick's G'_{ST} values for SNP loci and number of loci under stabilizing (below diagonal) and divergent (above diagonal) selection based on 99.9% neutral microsatellite marker confidence interval ranges for each pairwise comparison. Values in the brackets show percentage values to correct for different number of SNP loci data available for each comparison. In some pairwise comparisons, it was not possible to detect stabilizing selection (shown by 'UD=Undetermined' loci) due to the neutral microsatellite range spanning into negative values. This heatmap was created using 'diveRsity' package (Keenan et al., 2013) in R. See Table 2.2 for description of sampled population codes.



Appendix A9: Histogram showing patterns of divergence among six sampled snow bunting (*Plectrophenax nivalis*) populations for 101 SNP markers among the seven broad putative gene function categories. The Y-axis shows proportion of the pairwise population comparisons (n=1286) pairwise per-SNP locus Hedrick's G'_{ST} values. Pairwise comparisons at each SNP locus are determined to be under genetic drift or selection (stabilizing or divergent) based on pairwise G'_{ST} value relative to the 99.9% neutral microsatellite marker confidence interval range. For some comparisons it was not possible to determine selection status due to the neutral microsatellite marker range spanning zero, those comparisons are not shown here.

Appendix A10: Gene function annotation for the SNP loci (from the comparative analysis across fifteen pairwise comparisons) that were divergent in at least one pairwise comparison from population genetic analyses of six *Plectrophenax nivalis* populations. All listed loci were under stabilizing selection for global comparison (i.e. across all six populations). The gene ontology and detailed function information is derived from Gene Ontology database (Gene Ontology Consortium) and UniProt database.

Primer Name	Gene Description	Gene Ontology	Detailed Description
SNP_10	Serine/threonine-protein kinase LATS2	- Protein serine/threonine kinase activity	- Resulting protein aids in spindle formation during mitosis - Responds to cytoskeleton damage - Co-repressor of androgen-responsive gene expression
SNP_41	Activin receptor type-2A	- Positive regulation of protein phosphorylation - Activin activated receptor activity	- Encodes for receptor that mediates induction of adipogenesis by Growth Differentiation Factor 6
SNP_105	Ankyrin repeat and LEM domain-containing protein 2	- Protein phosphatase regulator activity - Mitotic nuclear membrane reassembly - Central nervous system development - Negative regulation of apoptotic process	- Involved in brain development
SNP_56	Activated CDC42 kinase 1	- Protein serine/threonine kinase activity - Transmembrane receptor protein tyrosine kinase activity	- Involved in cell spreading, migration, survival and cell growth, - May be involved in adult synaptic function and plasticity in brain development
SNP_175	Protocadherin gamma-C5	- Synapse organization - Integral component of plasma membrane - Calcium ion binding	- Involved in establishment and maintenance of specific neuronal connections in the brain
SNP_60	Receptor-type tyrosine-protein phosphatase zeta	- Protein tyrosine phosphatase activity - Transmembrane receptor protein	- Required for normal differentiation of precursor cells into

		tyrosine phosphatase activity - Integrin binding	mature myelinating oligodendrocytes - May play a role in establishment of contextual memory and learning
SNP_140	Transcription regulator protein BACH2	- DNA-binding transcription factor activity, RNA polymerase II-specific	- Protects cells by inducing apoptosis in response to oxidative stress, - Regulates adaptive immunity - Crucial for maintenance of regulatory T-cell function and B-cell maturation

Appendix B: Supplemental Material for Chapter 3

Appendix B1: Output from the first-stage Principle Component Analysis (PCA) representing variation in five male quality categories (Male State, Song Quality, Wing Pattern, Plumage Quality and Territory Quality) in male snow buntings *(Plectrophenax nivalis)* breeding at Mitivik Island, Nunavut, Canada. Bolded values indicate variables that loaded strongly onto principle component factors. * Represents a Box-Cox transformed term (please see 'Statistical analyses' section under Methods for more detail).

Quality measurements	**PCA loadings**		**Variable description**
Male State PCA	PC1: Arrival Body Mass and Testosterone	PC2: Arrival Physiological Health	
Variance explained (%)	36.80	32.44	
Eigenvalue	1.56	1.21	
Male arrival body mass	**0.87**	-0.04	Male body mass at the time of arrival.
Male arrival testosterone*	**0.84**	0.15	Concentration of plasma testosterone at the time of arrival.
Male arrival oxidative status	0.01	**0.82**	Oxidative balance: ratio of reactive oxygen metabolites to antioxidants at the time of arrival.
Male arrival plasma IgY	0.07	**0.78**	Levels of plasma immunoglobulin Y (IgY) serum proteins at the time of arrival.
Song Quality PCA	PC1: Song Structure	PC2: Song Complexity	
Variance explained (%)	50.51	46.58	
Eigenvalue	2.17	1.72	
Note duration	**0.98**	-0.06	Sum of note lengths in a song.
Song length	**0.98**	-0.04	Time elapsed from the start to the end of a song.
Syllable repetition	0.13	**0.98**	Number of times a specific element or cluster of elements observed on a spectrogram of a complete song OR the total number of unique syllables produced across a sample of 10 songs

Song versatility	-0.26	**0.95**	Number of unique syllables in a sample of 10 songs divided by the total syllables in the same sample of 10 songs
Wing Pattern PCA	PC1: Wing Spotting	PC2: Wing Patterns	
Variance explained (%)	47.07	43.47	
Eigenvalue	2.16	1.46	
Area of spots	**0.97**	-0.03	Total area of each spot within the white area of the wing.
Average spot size	**0.95**	-0.16	Average size of individual spots within the white area of the wing.
Area of extremity	-0.01	**0.94**	Area of black primary tips at the end of the white wings.
Area of alula	-0.17	**0.91**	Area of the black alula feathers on the wrist of the wing.
Body Plumage PCA	PC1: Mantle Plumage	PC2: Breast Plumage	
Variance explained (%)	40.61	31.78	
Eigenvalue	3.04	1.30	
Breast brightness	0.28	**0.60**	Mean reflectance of white breast feathers from 300-700 nm.
Breast UV chroma	0.38	**-0.88**	Mean reflectance of white breast feathers from 300-400 nm.
Breast saturation	-0.40	**0.84**	Intensity of the colour measured by maximum reflectance divided by mean reflectance of the white breast feathers.
Mantle brightness	**0.63**	-0.02	Mean reflectance of the black mantle (back) feathers from 300-700 nm.
Mantle UV chroma	**0.94**	-0.16	Mean reflectance of the black mantle (back) feathers from 300-400 nm.
Mantle saturation	**0.87**	-0.17	Intensity of the colour measured by maximum reflectance divided by mean reflectance of the black mantle feathers.

Territory Quality PCA	PC1: Territory Quality	
Variance explained (%)	68.90	
Eigenvalue	1.38	
Territory size	**0.83**	The total area of a male's breeding territory.
Rock cover	**-0.83**	Proportion of rock cover within a 5m radius of the male's primary social nest.

Appendix B2: Descriptions and interpretations of each principle component output from the first-stage Principle Component Analysis (PCA) representing variation in Male State, Song Quality, Wing Pattern, Body Plumage and Territory Quality in male snow buntings *(Plectrophenax nivalis)* breeding at Mitivik Island, Nunavut, Canada.

Male quality trait	Principle component	Description and interpretation
Male State	Arrival Body Mass and Testosterone	A high positive value denotes higher body mass and plasma testosterone levels at the time of arrival.
	Arrival Physiological Health	A high positive value denotes higher circulating levels of IgY and higher levels of oxidative stress.
Song Quality	Song Structure	A high positive value denotes longer songs with longer notes within the song.
	Song Complexity	A high positive value denotes songs with larger syllable repertoire and higher versatility.
Wing Pattern	Wing Spotting	A high positive value denotes larger proportion of the white area on an individual's wing that are covered in spots.
	Wing Patterns	A high positive value denotes larger black primary tips and larger alula on an individual's wing.
Body Plumage	Mantle Plumage	A high positive value denotes higher brightness, UV chroma and saturation of the black mantle (back) plumage.
	Breast Plumage	A high positive value denotes higher brightness and saturation, and lower UV chroma of the white breast plumage.
Territory Quality	Territory Quality	A high positive value denotes a larger breeding territory with a smaller proportion of rock cover around the nest.

Appendix B3: Output from the second-stage Principle Component Analysis (PCA) tests combining Male State, Song Quality, Wing Pattern, Body Plumage and Territory Quality components from first-stage PCA in male snow buntings *(Plectrophenax nivalis)* breeding at Mitivik Island, Nunavut, Canada. Bolded values indicate variables that loaded strongly onto principle component factors.

Quality measurements	**PCA loadings**			
Second-stage PCA	PC1	PC2	PC3	PC4
Variance explained (%)	28.32	20.35	17.15	14.86
Eigenvalue	3.16	1.77	1.29	1.03
Arrival Body Mass and Testosterone	-0.17	**-0.77**	0.20	0.15
Arrival Physiological Health	**0.86**	0.03	0.27	0.19
Song Structure	0.18	0.24	**0.81**	0.16
Song Complexity	0.05	0.09	-0.08	**0.91**
Wing Spotting	-0.06	0.34	**-0.79**	0.30
Wing Patterns	**0.94**	0.20	0.10	-0.08
Mantle Plumage	**-0.74**	-0.49	0.16	-0.07
Breast Plumage	0.11	**0.83**	0.28	0.23
Territory Quality	**0.56**	-0.29	0.15	0.52

Appendix B4: Descriptions and interpretations of each principle component output from the second-stage Principle Component Analysis (PCA) representing variation in Male State, Song Quality, Wing Pattern, Body Plumage and Territory Quality in male snow buntings *(Plectrophenax nivalis)* breeding at Mitivik Island, Nunavut, Canada.

PC combination	Principle component	Description and interpretation
PC1	Wing Patterns	A high positive value denotes larger black primary tips and larger alula on an individual's wing.
	Arrival Physiological Health	A high positive value denotes higher circulating levels of IgY and higher levels of oxidative stress.
	Territory Quality	A high positive value denotes a larger breeding territory with a smaller proportion of rock cover around the nest.
	Mantle Plumage	A high positive value denotes lower brightness, UV chroma and saturation of mantle plumage.
PC2	Arrival Body Mass and Testosterone	A high positive value denotes lower body mass and plasma testosterone levels at the time of arrival.
	Breast Plumage	A high positive value denotes higher brightness and saturation, and lower UV chroma of the white breast plumage.
PC3	Wing Spotting	A high positive value denotes smaller proportion of the white area on an individual's wing that are covered in spots.
	Song Structure	A high positive value denotes longer songs with longer notes within the song.
PC4	Song Complexity	A high positive value denotes songs with larger syllable repertoire and higher versatility.

Appendix B5: Maternity assignments for 25 snow bunting (*Plectropnenax nivalis*) chicks from the 2010 breeding season at Mitivik Island (Nunavut, Canada) with low pair-LOD scores. Bold refers to the chicks that were not assigned to their social mothers after the maternity analysis.

Nest	Offspring ID	Social mother ID	Pair LOD score (offspring-social mother)	CERVUS-assigned mother ID	Pair LOD score (offspring-CERVUS assigned mother)
1	c1.1	N	-6.43	N	-6.43
	c1.2	N	-4.18	N	-4.18
	c1.4	N	-4.18	N	-4.18
	c1.5	N	-1.74	N	-1.74
	c1.6	N	-5.48	N	-5.48
2	**c2.5**	3	-5.73	25	-0.74
4	**c4.1**	16	-1.63	N	-0.73
	c4.4	16	-5.28	18	0.03
7	c7.1	R	-6.19	R	-6.19
	c7.4	R	-4.21	R	-4.21
	c7.5	R	-4.27	R	-4.27
8	c8.4	25	-6.71	25	-6.71
9	c9.1	Q	-3.92	Q	-3.92
10	**c10.4**	D	-6.71	14	-1.25
11	**c11.1**	14	-1.06	S	2.85
12	c12.2	O	-1.77	O	-1.77
	c12.5	O	-8.92	O	-8.92
13	**c13.4**	18	-7.29	Q	-5.54
16	**c16.1**	V	-5.22	K	-1.99
17	**c17.1**	I	-6.59	D	-5.28
	c17.2	I	-6.34	S	-1.11
	c17.3	I	-3.77	S	1.46
18	c18.2	17	-4.93	17	-4.93
	c18.3	17	-4.97	17	-4.97
	c18.4	17	-2.61	17	-2.61

Appendix B6: Reproductive success matrices for snow bunting (*Plectrophenax nivalis*) males breeding at Mitivik Island, Nunavut, Canada.

Nest	Male ID	Male Band ID	Year	Within-pair Reproductive Success (WPRS)	Extra-pair Reproductive Success (EPRS)	Total Reproductive Success (TRS)	# of Nests with At Least 1 Extra-pair Young (EPRS Allocation)
1	F	2341-92463	2010	6	5	11	4
2	E	2291-39973	2010	3	3	6	1
4	24	2341-92787	2010	3	1	4	1
5	C	2261-83063	2010	2	2	4	2
6	2	2341-92624	2010	1	0	1	0
7	L	2341-92398	2010	5	0	5	0
8	11	2341-92774	2010	4	1	5	1
9	G	2341-92399	2010	0	6	6	4
10	4	2341-92670	2010	3	1	4	1
11	B	2261-83112	2010	5	0	5	0
12	J	2261-83187	2010	4	1	5	1
13	12	2341-92775	2010	5	5	10	3
14	U	2341-92464	2010	0	1	1	1
15	M	2341-92378	2010	N/A	1	N/A	1
16	T	2341-92438	2010	4	0	4	0
17	A	2291-39982	2010	5	1	6	1
18	31	2341-92794	2010	4	2	6	2
1	144	2341-93049	2011	4	0	4	0
2	#A	2341-92398	2011	4	5	9	1
3	#B	2341-92378	2011	1	0	1	0
4	168	2341-93151	2011	1	1	2	1
5	#F	2341-92854	2011	5	3	8	3
6	178	2341-93171	2011	4	0	4	0
7	135	2341-93033	2011	3	4	7	4
8	#H	2341-92464	2011	1	0	1	0
9	176	2341-93163	2011	1	0	1	0
10	184	2341-93178	2011	2	1	3	1
11	186	2341-93180	2011	3	0	3	0
12	185	2341-93179	2011	0	1	1	1
13	#G	2341-92440	2011	0	6	6	4

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