Doctoral theses at NTNU, 2024:105

Doctoral thesis

Lisa Dickel

Multi-generational genetic and fitness effects of immigration within a natural meta-population of song sparrows (*Melospiza melodia*)

NTNU
Norwegian University of Science and Technology
Thesis for the Degree of
Philosophiae Doctor
Faculty of Natural Sciences
Department of Biology

NTNU
Norwegian University of
Science and Technology

Lisa Dickel

Multi-generational genetic and fitness effects of immigration within a natural meta-population of song sparrows *(Melospiza melodia)*

Thesis for the Degree of Philosophiae Doctor

Trondheim, April 2024

Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

NTNU
Norwegian University of
Science and Technology

NTNU
Norwegian University of Science and Technology

Thesis for the Degree of Philosophiae Doctor

Faculty of Natural Sciences
Department of Biology

ISBN 978-82-326-7804-4 (printed ver.)
ISBN 978-82-326-7803-7 (electronic ver.)
ISSN 1503-8181 (printed ver.)
ISSN 2703-8084 (online ver.)

Doctoral theses at NTNU, 2024:105

Printed by NTNU Grafisk senter

Summary

Fragmentation leads to smaller habitat patches and population sizes, endangering population fitness and persistence, including through inbreeding depression and loss of genetic diversity. These effects can be mitigated through immigration when immigrants contribute to replenishing local genetic diversity, while also reducing local inbreeding and the resulting expression of inbreeding depression. The concept of genetic rescue relies on these effects in its theoretical predictions and management practice. However, there are also potential negative consequences of immigration, such as loss of local adaptation. In practice, the multi-generational genetic effects of natural immigration, stemming from the fitness of immigrants and their descendants, are not yet well known, precluding informed inferences on the net consequences.

Hence, in this thesis, I quantified key multi-generational effects of immigration using long-term data from a system of song sparrows, *Melospiza melodia*, on Mandarte Island, BC, Canada, where the focal population occasionally receives natural immigrants. Specifically, I tested standard assumptions concerning the relatedness of immigrants to the recipient population (**paper I**), the fitness consequences of immigration across successive generations (**paper II**), consequential introgression of immigrant genes in the population (**paper III**) and potential modulation of such fitness and introgression consequences through non-random mating (**paper IV**).

Specifically, **in paper I**, I tested the standard assumptions of population genetics, that immigrants are outbred and unrelated to the recipient population at the time of arrival. Using both microsatellites and pedigree inbreeding coefficient *f*, I showed that immigrants are effectively unrelated to each other and to the local population, and are also outbred relative to the local scale of pedigree inbreeding. These results show that recent immigrants have potential to import novel alleles, decrease local inbreeding, and induce substantial fitness consequences.

In **paper II,** I quantified the relative fitness of immigrants, natives and their descendants, utilizing line cross theory and planned comparisons to quantify key differences among defined filial groups for multiple major fitness components. I found strong effects, including high relative fitness in the first generation of immigrant-native descendants (F1), and loss of fitness in the second generation (F2), indicating strong heterosis followed by epistatic breakdown. These results indicate strong non-additive genetic effects on fitness, and imply that risks of outbreeding depression in crosses between weakly diverged natural populations should not be ignored.

In **paper III,** to quantify the expected persistence of immigrants' genes in the focal population, collaborators and I used long-term pedigree data to estimate genetic contributions of immigrants and natives across up to fifteen years from arrival and recruitment. We showed that female immigrants contributed more than natives, while male immigrants' lineages went typically rapidly extinct. Further, the high genetic contribution of female immigrants only emerged several years after the immigrants arrived, consistent with heterosis-enhanced introgression.

Finally, in **paper IV,** I tested whether the fitness consequences of immigration were modulated through non-random pairing, and specifically whether F1 individuals paired with natives rather than other F1 individuals, thereby avoiding producing low-fitness F2 offspring. I found that low pairing success of male immigrants was a mechanism underpinning their fitness, with no evidence that F1s avoided each other.

In conclusion, immigrants had major fitness effects, both positive, and negative, and immigrants' genes persisted over longer ecological time frames (here, 15 years), with strong sex-specific effects. These are surprisingly strong consequences of immigration, given that the focal song sparrow population is relatively well-connected and apparently unlikely to be strongly diverged from surrounding populations. These findings will be relevant to ambitions to

understand and predict net genetic and fitness effects of immigration, and help to disentangle the trade-off between the benefits of outbreeding and the loss of local adaptation. Thereby, they can inform genetic rescue considerations and provide conceptual frameworks for estimating and understanding such complex effects in further systems in nature.

Sammendrag

Ødeleggelse og fragmentering av habitatet til en art fører til mindre leveområder og mindre bestandsstørrelse, hvilket kan gjøre at bestander står i fare for a bli utryddet. Utryddelse kan i slike tilfeller skje blant annet gjennom genetiske effekter som innavlsdepresjon og tap av genetisk mangfold. Disse negative effektene kan reduseres gjennom innvandring, hvor immigranter kan redusere innavl og ekspresjon av innavlsdepresjon, samt øke genetisk variasjon. Samtidig kan innvandring også gi negative effekter, for eksempel dårligere tilpasning til det lokale miljøet. Til tross for at kunnskap om dette er svært viktig siden både natur og habitater som arter lever i ødelegges av menneskeheten, er det fortsatt for lite kunnskap om netto-effektene av innvandring i naturlige bestander.

I ph.d.-avhandlingen min brukte jeg data fra en bestand av sangspurv, *Melospiza melodia*, som lever på en liten øy på vestkysten av Canada, til å undersøke spurvenes overlevelse og reproduksjon («fitness») over flere generasjoner etter innvandring til bestanden. Sangspurver fra omkringliggende øyer kan periodevis immigrere til studiebestanden, hvilket kan forhindre bestanden i å dø ut. Jeg undersøkte om immigrantene var i slekt med sangspurvene i studiebestanden (artikkel I), hva de evolusjonære fitness-konsekvensene av immigrasjon var (artikkel II), om immigrantene introduserte

ny genetisk variasjon, og hvor lenge det nye genetiske bidraget holdt seg i bestanden (artikkel III), og til slutt om fitness-effektene og det genetiske bidraget fra immigrantene var forårsaket av makevalg og ikke-tilfeldig paring (artikkel IV).

Resultatene mine viste at sangspurv-immigranter er hverken i slekt med sangspurvene i studiebestanden, eller med hverandre. Jeg fant også store fitness-forskjeller mellom lokale sangspurver, immigranter, og etterkommerne deres: Avkom med en immigrant- og en lokal forelder («hybrider») hadde veldig høy fitness, mens avkom etter to slike «hybrid»-foreldre hadde betydelig lavere fitness. Jeg fant også ut at immigranter, og spesielt hunn-immigrantene, bidro med mer ny genetisk variasjon til bestanden enn forventet målt over lengre tidsrom (ca. 15 år). Til slutt fant jeg at paringspreferanser kunne forklare noen av disse effektene.

Disse funnene viser sterke og komplekse effekter, som var sterkere enn forventet for populasjoner som er ikke veldig sterkt innavlet sammenlignet med mange populasjoner av bevaringsrelevans. Resultatene er overraskende og kritiske for forståelsen av viktige prosesser innen evolusjonsbiologi og økologi. Jeg viser at det både kan være positive og negative effekter av innvandring i bestander i naturen. Denne kunnskapen kan hjelpe oss å forvalte fragmenterte bestander, for eksempel gjennom flytting av individer for å forhindre utryddelse av små bestander som man ønsker å bevare.

Acknowledgments

I am very grateful for the past years of this PhD, and there are many people I want to thank for their contributions to making these so enjoyable.

First, I am grateful for all of my supervisors who guided me through this work. Jane, thank you for all of your dedication to introduce me to a whole range of topics which were fascinating, and yet also unfamiliar to me. I am very grateful for the opportunity to learn from you, it was really a pleasure and you are an amazing mentor.

Henrik, Peter, Lukas, and Pirmin, I want to thank all of you for a great collaboration across multiple countries and even continents during the last years.

Peter, thank you for bringing me along for the memorable and fieldwork experience on Mandarte. Peter, Megan and Taise, thank you for making the time on Mandarte so enjoyable and for ensuring my survival on boats and slippery rocks. It was truly special to work in a study system that exists for much longer than I do. I am grateful for all the people who worked hard for the creation of this long-term data set and made so many fascinating insights into evolution and population dynamics possible, including the continued and ongoing efforts. Especially, I also want to especially thank the Tsawout and Tseycum First Nations for allowing access to X̱OX̱ DEŁ (Mandarte island) during all of these years.

I also want to thank all my colleagues at CBD and IBI, this has been an inspiring and stimulating environment to learn. Debora, you have been such a dedicated co-author, mentor and friend with a very good eye for every detail. Cassandra, Astrid, Laura, Ragnhild, Rune, Safa, Maris and Bert, thank you for both, encouragement to work and to taking breaks, and for making this time so enjoyable. Astrid, thank you for being my writing partner who saw most of this thesis come together from up close. Sarah and Ellen, I appreciated you very much as office mates, it was a joy seeing you succeed and learning from you. I am excited to see what all of our futures will bring.

Further, I want to thank my previous and ongoing mentors who were immensely helpful to discover the next scientific adventure and especially helped me to persist in my work over the last 10 years. Olivier and Jeremy, Morten and Barbara, thank you for all of your support that helped me to even get started on this PhD journey. Chasing spiders and cattle with you in forests and marshlands, as well as chasing coding errors in R turned out to be amazing opportunities for thinking, discussing and developing my academic interests.

Erik, thank you for all the visits, walks, and seamless emotional and academic support. Milena, Charis, Milena, Sus and Milan, thank you for your ongoing help to figure out what is the right direction to head to, even from far, all of you have been an amazing compass for many years. Jack, Einy and Ola, Ole-Henrik, and Jelena, thank you for all the good memories, you made coming to Norway very enjoyable.

I am also grateful for the amazing group of people I got to know through what started as a temporary science communication project during the pandemic, and has grown to be a regular journal club running for already 3 years. I have learned a lot from all of you and our discussions. Thank you, Lisbeth (and many others), for your encouragement that helped to overcome my hesitation and expand this project to what it is now.

Lyuba and Marianne, thank you for your presence in my life and for your humor, with which you help me think and be critical in constructive ways. You have been a strong support through both difficulties and successes.

I also want to thank my family for accompanying me through all the adventures of my education as a biologist, which included some unexpected turns and also dispersal. Rudolf and Elvira, Irina and Anahita, Thomas, Andreas, Wolfgang and Christa, you all contributed in your own ways. Wilfried, you have always been and will continue to be an inspiration with your thoughtfulness and curiosity for both nature and humans, you are missed.

Finally, I am very grateful for my Trondheim family, Rune and Nyako, thank you for making this place my home, even during my “deadline mode”. In addition, I also gained an extended family here: Ingvill and Tore, thank you so much for all of your help in the last years, I am very lucky to have you.

All of you (and many more people of which some I will have forgotten, my apologies!) have made this latest adventure possible, and importantly, a great joy, thank you so much.

Contents

Summary....................1
Sammendrag....................3
Acknowledgments....................5
Contents....................7
List of articles....................9
Declaration of contributions....................10
Introduction....................11
Aims....................21
General Methods....................22
Paper summaries....................30
Discussion & Future Directions....................35
Literature cited....................39

Paper I - IV

List of articles

This thesis contains the following articles:

I. **Dickel, L.**, Arcese, P., Nietlisbach, P., Keller, L. F., Jensen, H., & Reid, J. M. (2021). Are immigrants outbred and unrelated? Testing standard assumptions in a wild metapopulation. Molecular Ecology, 30:5674-5686. https://doi.org/10.1111/mec.16173

II. **Dickel, L.**, Arcese, P., Keller, L. F., Nietlisbach, P., Goedert, D., Jensen, H., Reid, J. M. (2024). Contrasting multi-generational fitness effects of natural immigration indicate strong heterosis and epistatic breakdown in a wild bird population. *In press.* The American Naturalist.

III. Reid, J. M., **Dickel, L**., Keller, L. F., Nietlisbach, P., Arcese, P. (2024). Multi-generation genetic contributions of immigrants reveal cryptic elevated and sex-biased effective gene flow within a natural meta-population. *In press.* Ecology Letters.

IV. **Dickel, L.,** Goedert, D., Keller, L. F., Arcese, P., Reid, J. M. Does non-random mating modulate the multi-generational fitness effects of immigration? *Manuscript*.

Declaration of contributions

I: Lisa Dickel designed and undertook the analyses and drafted the manuscript in collaboration with Jane M. Reid. Peter Arcese undertook and oversaw long-term field data collection. Pirmin Nietlisbach and Lukas F. Keller led genotyping and pedigree reconstruction. Pirmin Nietlisbach, Lukas F. Keller and Jane M. Reid contributed to fieldwork. All authors contributed substantially to conceptual development and manuscript editing.

II: Lisa Dickel and Jane M. Reid designed and performed the analyses and drafted the manuscript. Peter Arcese undertook and oversaw data collection to create and maintain the pedigree. Pirmin Nietlisbach and Lukas F. Keller led genotyping and reconstruction of the genetic pedigree. Pirmin Nietlisbach, Lukas F. Keller and Jane M. Reid contributed to fieldwork. Debora Goedert contributed to conceptual synthesis. All authors contributed substantially to conceptual development and manuscript editing.

III: Jane M. Reid designed and undertook the analyses and drafted the manuscript in collaboration with Lisa Dickel. Peter Arcese undertook and oversaw long-term field data collection. Pirmin Nietlisbach and Lukas F. Keller led genotyping and pedigree reconstruction. Pirmin Nietlisbach, Lukas F. Keller and Jane M. Reid contributed to fieldwork. All authors contributed substantially to conceptual development and manuscript editing.

IV: Lisa Dickel designed and undertook the analyses and drafted the manuscript. Jane M. Reid and Debora Goedert contributed to conceptual development, and guidance on analyses and writing. Lukas F. Keller and Peter Arcese overtook and oversaw long-term field data collection. All authors contributed to conceptual development and manuscript editing.

Introduction

Metapopulations in the context of global change

Habitat fragmentation is one of the key processes underlying the current global biodiversity crisis, leading to populations disintegrating into smaller sub-populations (Haddad et al., 2015). The persistence of resulting small populations can be threatened by, among others, genetic processes, such as inbreeding and inbreeding depression, drift, and resulting loss of adaptability to changing conditions (Keller and Waller, 2002; Carlson et al., 2014).

However, many species have adapted to living in smaller populations connected by dispersal (i.e. metapopulations; Hanski and Simberloff, 1997) and have evolved mechanisms reducing the prevalence of inbreeding, through both mate choice and dispersal (Szulkin and Sheldon, 2008; Szulkin et al., 2013). For instance, island systems are naturally fragmented, and have led to critical insights into metapopulation dynamics (Macarthur and Wilson, 1967). Metapopulations are defined as local and distinct breeding populations which are connected by dispersal (Hanski and Simberloff, 1997). These systems can aid our understanding of the mechanisms and processes acting in anthropogenically fragmented metapopulations (Haila, 2002). Dispersal is movement of individuals with potential resulting gene flow, if individuals succeed to immigrate and reproduce (e.g. Kardos et al., 2018; Saastamoinen et al., 2018; Martinig et al., 2020; Åkesson et al., 2022). Hence, dispersal can potentially rescue fragmented populations.

Consequences of immigration can be variable, depending on characteristics and numbers of immigrants, as well as characteristics of the recipient population, including genetic and ecological distance between populations, selection pressures and mating system (e.g. Pickup et al., 2019). For an individual, dispersal is a major decision point of their life history which can have both major costs or benefits to their fitness (Edelaar and Bolnick, 2019; Mobley et al., 2019; Barbraud and Delord, 2020; Martinig et al., 2020). Beyond individual consequences, immigration can result in various critical demographic and evolutionary consequences for populations (Marr et al., 2002; Millon et al., 2019; Fitzpatrick et al., 2020). These combine both potentially positive and negative fitness consequences which can occur simultaneously or in sequence; for example immigrants could reduce inbreeding while simultaneously increasing maladaptation, resulting in a trade-off between costs and benefits to fitness (Fitzpatrick and Reid, 2019; Reid et al., 2021, Fig. 1).

Consequently, the genetic effects of dispersal and resulting immigrants on fitness and populations persistence are now recognized as being critical in conservation applications (Keller and Waller, 2002; Bell, 2013; Fitzpatrick et al., 2016; 2020). However, still relatively little is known about the net effects of immigration in meta-populations in an eco-evolutionary context (Bell et al., 2019). While net effects are still challenging to predict, I will briefly summarize relevant evidence and theory in the following part of this thesis, and novel empirical evidence will follow in the included papers (I-IV).

Potential consequences of immigration

Immigrants can have positive effects on population demography and fitness, inducing possibly three different types of „rescue“ to populations which might otherwise face reduced fitness (Carlson et al., 2014). First, immigrants can increase the population size directly, referred to as demographic rescue (Derry et al., 2019; Millon et al., 2019). Further, when immigrants succeed to mate in an inbred recipient population, they can alleviate inbreeding and resulting expression of inbreeding depression, referred to as genetic rescue. To induce this type of rescue, individuals are often translocated because natural dispersal in such scenarios is often not possible or not sufficient (Frankham, 1998; Tallmon et al., 2004; Bell et al., 2019). In addition to alleviating inbreeding, outbreeding can increase fitness in offspring of unrelated parents by inducing heterosis through reversal of genetic drift (Charlesworth and Willis, 2009). Hence, heterosis can even enhance fitness of offspring between unrelated parents beyond the fitness of both parents, before these effects typically decline again in later generations (Mather and Jinks, 1977). Finally, immigrants can replenish genetic variation which can then facilitate adaptations to changing environments, which is referred to as evolutionary rescue in the case where evolution reverses a population decline (Gomulkiewicz and Shaw, 2013; Carlson et al., 2014; Hufbauer et al., 2015; Bell et al., 2019).

Conversely to such effects that are the primary focus in the context of conservation, immigrants can also have negative effects on fitness. They might cause the loss of local adaptation and thereby cause maladaptation leading to lower fitness (Pärssinen et al., 2020; but see Fitzpatrick et al., 2020). Depending on the strength of selection acting on the maladapted traits, and the migration load, a migration-selection balance can be reached, so that evolution stands still effectively through the balance of both negative effects of immigration and positive effects of local adaptive evolution (Lenormand, 2002; Garant et al., 2007; Bolnick and Nosil 2007; Reid et al 2021, Fig. 1).

Local adaptation is primarily linked to heritable (i.e. additive genetic) effects. However, introgression of maladaptive genetic variants might be facilitated through initial heterosis in immigrants’ offspring („heterosis-enhanced introgression“), sometimes also referred to as „genetic swamping”, when maladaptive genetic variants are introduced and cannot be counteracted by selection (Rhymer and Simberloff, 1996; Allendorf et al., 2004). Hence, such initially increased fitness might thereby have net negative consequences, if it leads to loss of local adaptation, while initial fitness benefits of heterosis are lost in later generations (Mather and Jinks, 1977). Yet, local adaptation can also persist despite high frequency of immigration (Fitzpatrick et al., 2020). Loss of local adaptation can also result from epistatic effects through the loss of co-adapted gene complexes, which become apparent when recombination occurs, and hence might not yet be manifested in the F1 generation (Kawecki and Ebert., 2004; Fig. 1).

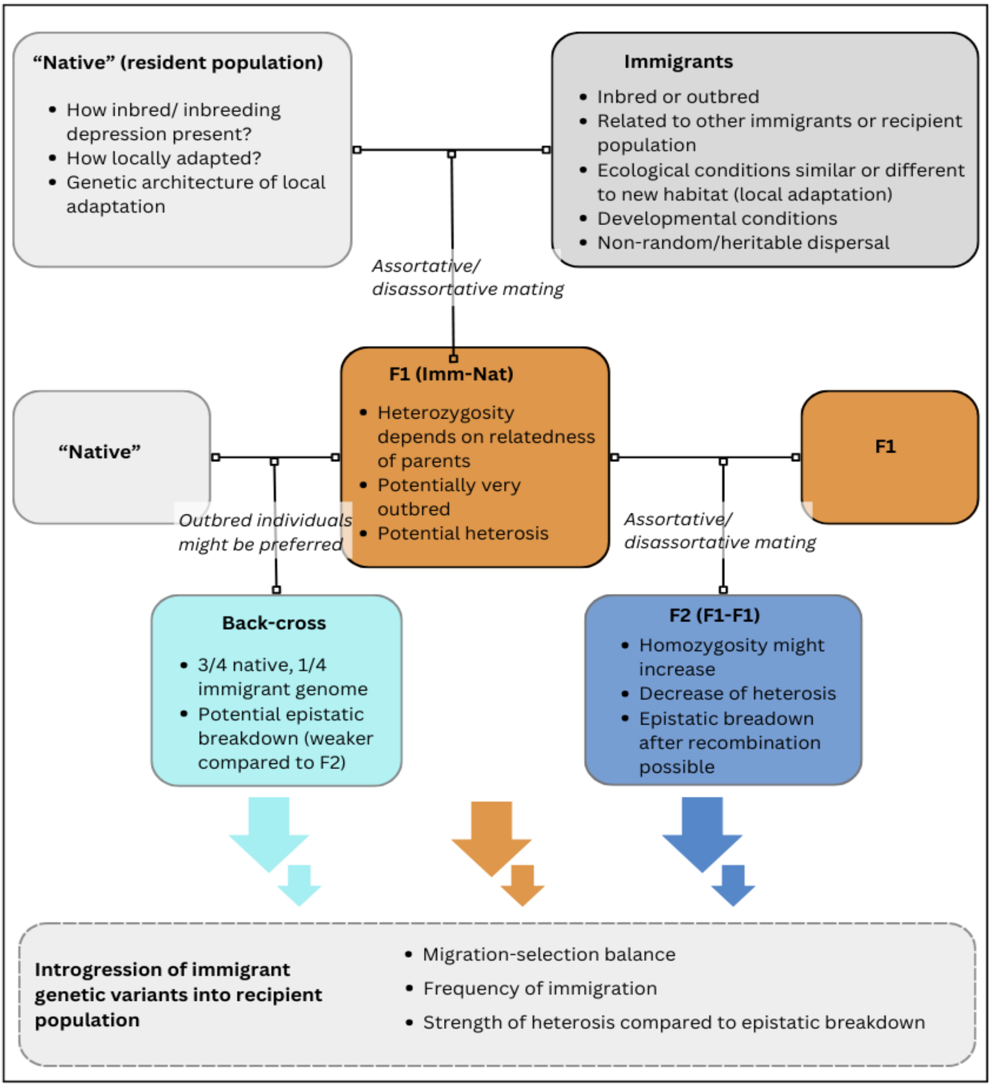
Thereby, heterosis, and/or reduction of the expression of inbreeding depression occurring following immigration might positively impact population fitness, while the simultaneous loss of locally adapted gene complexes might negatively impact population fitness (Hoffmann et al., 2021). Then, net consequences and effective introgression will depend on the whole complex network of additive, and non-additive genetic effects, as well as realized frequencies of specific crosses in the wild (Millon et al., 2019), which then can be mediated by mating frequencies. This complexity of consequences following immigration underlies ongoing debates about the genetic consequences of immigration (Gomulkiewicz and Shaw, 2013).

Adding complexity, not all immigrants are equal in their effects. Individual characteristics of immigrants are relevant for their survival probability and reproductive success when arriving into a new population. Dispersal might be heritable or non-random, then potentially reducing the effective positive effect on local fitness if immigrants' descendants are more likely to disperse again (Doligez and Pärt, 2008; Edelaar and Bolnick, 2012; Saastamoinen et al., 2017). Because immigrants' ancestors are typically not part of the local pedigree, if it does not encompass an entire meta-population system, immigrants are commonly assumed to be unrelated and outbred relative to the local population (Ballou, 1983; Pemberton, 2008). If those assumptions are not met, predictions for genetic and fitness consequences of immigration can be altered. How related immigrants are to each other and the recipient population, and how inbred they themselves are, can impact how strong genetic and fitness consequences following outbreeding might be (e.g. Hammerly et al., 2016, Fig. 1). Additionally, how different the ecological conditions of those environments are might impact the degree of mismatch with the environment, and therefore whether maladaptation might reduce fitness for arriving immigrants and their descendants (Kawecki and Ebert, 2004; Bolnick and Nosil, 2007; Frankham et al., 2011; Svensson et al., 2017; Svensson et al., 2018).

Mating systems might modulate frequency of specific crosses, and thereby gene flow and population fitness consequences following immigration. While mating systems are frequently linked to the mitigation of inbreeding depression and the loss of local adaptation, both are rarely considered simultaneously (but see Epinat and Lenormand, 2009). Preference for specific phenotypes might occur through sexual selection (Anderson, 2004). Then, based on mechanisms such as assortative mating or inbreeding avoidance, mating systems might impact how successful immigrants are as mates after arrival in the recipient population (Epinat and Lenomard, 2009; Jiang et al., 2013; Szulkin et al., 2013, Fig. 1). Due to sexual selection and resource acquisition processes being frequently sex-specific, the sex of immigrants arriving into a recipient population can affect their demographic and evolutionary impacts (Martinig et al., 2020; Barbraud and Delord, 2020; Li and Kokko, 2019).

Mate choice might also be impactful not only for immigrants themselves, but also for their descendants. For example, descendants of immigrants might be preferred or avoided by the local population as mates, or they might show preference for pairing with each other, thereby potentially leading to segregation (Åkesson et al., 2016; Schmidt and Pfennig, 2016; Lamichhaney et al., 2018). Potential fitness consequences observed in specific immigrant descendants then could be either ameliorated or exacerbated through such mating preferences or avoidances affecting frequencies of crosses (Fig. 1).

Fig. 1: Conceptual figure highlighting key points of characteristics, fitness effects and potential relationships among individuals of classic groups of line cross theory. Specifically, it shows immigrants (dark grey), natives (local population, light grey) and their F1 (orange), F2 (dark blue) and backcross (light blue) descendants with their genetic and potential fitness characteristics (in boxes) and their potential relationships with each other through mating systems (in between boxes). The box with the dotted outline shows potential aspects affecting genetic introgression of immigrant genetic variants into the recipient population which can occur through such crosses.


"Native" (resident population)
How inbred/ inbreeding depression present?
How locally adapted?
Genetic architecture of local adaptation
Immigrants
Inbred or outbred
Related to other immigrants or recipient population
Ecological conditions similar or different to new habitat (local adaptation)
Developmental conditions
Non-random/heritable dispersal
Assortative/ disassortative mating
F1 (Imm-Nat)
Heterozygosity depends on relatedness of parents
Potentially very outbred
Potential heterosis
"Native"
Outbred individuals might be preferred
F1
Assortative/ disassortative mating
Back-cross
3/4 native, 1/4 immigrant genome
Potential epistatic breakdown (weaker compared to F2)
F2 (F1-F1)
Homozygosity might increase
Decrease of heterosis
Epistatic breadown after recombination possible
Introgression of immigrant genetic variants into recipient population
Migration-selection balance
Frequency of immigration
Strength of heterosis compared to epistatic breakdown

The knowledge gap between speciation and inbred lines

Fitness consequences after outbreeding between species are well described in the hybridization literature (Orr, 1995; Atsumi et al., 2021; Muraro et al., 2022), as are the consequences of crossing between highly inbred lines through line cross experiments (Mather and Jinks, 1977; Fenster and Galloway, 2000, Fig. 3). However, there are currently only few wild systems in which fitness consequences of natural immigration have been thoroughly examined (but see Marr et al., 2002 in song sparrows *Melospiza melodia*; Martinig et al., 2020 in red squirrels *Tamiasciurus hudsonicus*). Both theoretical and empirical work is needed to aid the understanding of this topic (Goedert et al., unpublished manuscript, Fig. 2), as well as its practical application.

The history of quantifying inbreeding consequences in the wild can shed some light on the sparsity of research on fitness consequences following natural dispersal. Initially, inbreeding depression was considered to be unlikely and irrelevant in wild populations. Then, in the late 20th century it was discovered that inbreeding is indeed reducing fitness in the wild, with inbreeding depression reported across multiple systems, and effects being even stronger than under laboratory conditions (Crnokrak and Roff, 1999; Keller and Waller, 2002). Genetic rescue through translocating individuals between populations became a more common practice and is widely advocated today (Frankham 2010; Ralls et al., 2018; Fitzpatrick et al., 2023). Similarly to inbreeding depression, outbreeding depression is widely known in both agricultural and experimental work of line cross theory, yet its existence or relevance in the wild are still questioned (Frankham et al., 2011; Ralls et al., 2018, Fig. 2). Outbreeding depression was considered relevant only where populations were either extremely inbred (as in laboratory lines), or where they were diverged for >500 years (Ralls et al., 2018). Therefore, outbreeding depression was not considered relevant for populations which are relatively connected through frequent dispersal, and/or experience similar environmental conditions (Frankham et al., 2011; Ralls et al., 2018).

Immigrants often make substantial demographic contributions to populations (Millon et al., 2019). Yet, fitness effects are rarely monitored for multiple generations after natural immigration, meaning that the net fitness of immigrants and their descendants are rarely estimated (but see Marr et al., 2002; Martinig et al., 2020). Systematic use of line-cross theory to unravel underlying genetic architectures is therefore currently an outstanding challenge (Goedert et al., unpublished manuscript). This thesis will present some conceptual and empirical advances to study effects of immigration within a natural metapopulation system.

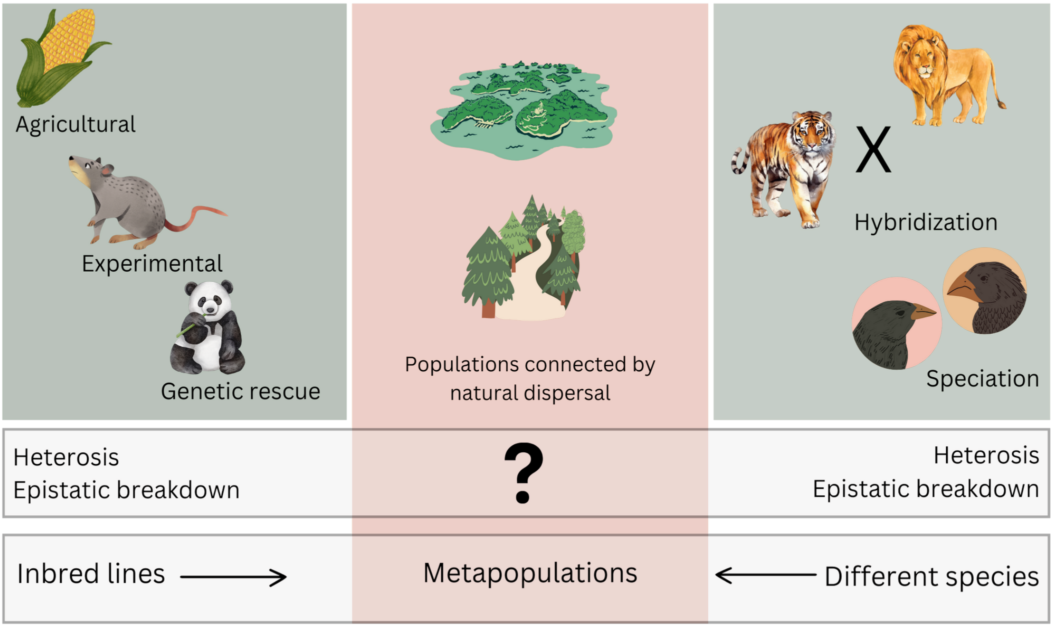


Fig. 2: Conceptual figure highlighting the knowledge gap of the consequences of immigration in meta-population contexts. This gap (middle panel) is embedded within empirical and theoretical work from inbred lines (left panel) and speciation/ interspecific hybridization literature (right panel). Heterosis and epistatic breakdown, for which there is evidence both after crossing inbred lines and species, have often been assumed to be irrelevant in natural meta-population context, but are now recognized as potentially important and require investigation.

Aims

The overall aim of this thesis is to advance the understanding of genetic, fitness and evolutionary consequences of immigration in a natural metapopulation, which I accomplished using up to 40 years of complete pedigree and life history data from a small population of song sparrows (*Melospiza melodia*) receiving occasional natural immigrants.

Specifically, **paper I**, tests the standard assumptions of evolutionary theory concerning whether immigrants are unrelated to each other, and outbred and unrelated compared to natives. This allows formulation of predictions for resulting fitness consequences.

Paper II tests specific predictions resulting from line-cross theory, specifically regarding the presence of heterosis and epistatic breakdown in fitness of immigrants' descendants.

Paper III quantifies expected genetic contributions of immigrants compared to natives, alongside time frames of such contributions for females and males.

Paper IV tests whether such fitness consequences and introgression patterns detected in papers II and III might be modulated by the mating system.

General Methods

To achieve the aims of this thesis, I required a study system with natural immigration with detailed pedigree and fitness data. Specifically, it was necessary to distinguish immigrants from resident individuals in the focal population, and further, a full pedigree was needed to track descendants across multiple generations after immigration. However, immigrants' ancestors are usually not part of the local pedigree, except if a pedigree encompasses a whole metapopulation. Therefore, immigrants are typically assumed to be unrelated and outbred (Fig. 1). Hence, in addition to a full pedigree, molecular data for locals and immigrants were needed to establish the relationship between pedigree inbreeding and marker homozygosity, thereby allowing interpreting immigrant inbreeding and relatedness on the local population pedigree scale (paper I).

Then, comparing fitness between locally hatched individuals and immigrants can be challenging because pre-dispersal fitness components for immigrants are typically lacking or not meaningful if they were measured in a different environment. This challenge can be overcome by using data on separate, fine-scaled fitness components, such that there can be meaningful comparisons for those components which are available for all individuals including immigrants (i.e. adult fitness components). As an additional advantage, potentially biased fitness components can then be isolated, which is especially relevant for local juvenile survival, which can be indistinguishable from emigration.

Island systems have been understandably popular to study effects of fragmentation and dispersal and metapopulation research, because here, habitat patches can be distinguished with clear boundaries, leading to clear definitions of local individuals and immigrants (MacArthur and Wilson, 1967, Hanski and Simberloff, 1997). I used such an island system, the song sparrow (*Melospiza melodia*) population of XOX DEŁ (English name used from here: Mandarte Island), BC, Canada (latitude 48.6329°, longitude -123.2859°, 0.06 km², Fig. 3), a population which has been studied intensely since the 1970s, where data on immigration are available. This system has already offered a lot of valuable insights into many key topics of evolutionary and population biology. These include inbreeding in the wild and the genetics underlying life history traits, especially considering immigration (Keller, 1998; Keller et al., 2001; Keller and Arcese, 1998; Reid et al., 2011; 2014; 2015; 2016; 2021; Reid and Sardell, 2012; Wolak et al., 2018; Reid and Arcese, 2020). Due to the available long-term data of this well studied population, lying within a natural metapopulation, it was very valuable for achieving the aims of this thesis.

Study species: the song sparrow

The song sparrow (*Melospiza melodia*) is a common, North American songbird species of the family Emberizidae with over 20 subspecies (Arcese et al., 2002). The species has a large distribution range throughout North America and inhabits a variety of habitats, including gardens, fields, natural grass and scrub lands, riparian habitats, and salt marshes (Nice, 1937; Marshall, 1948; Patten et al., 2004).

Song sparrows vary in their morphology, such as size and plumage, and life history with variation in migratory behavior dependent on the climate (Arcese et al., 2002; Chan and Arcese, 2003). Recently, local adaptation on a relatively small spatial scale in a song sparrow metapopulation system in California was demonstrated, where some song sparrows had increased tolerance to salt water likely as an adaptation to life in salt marshes (Walsh et al., 2019). Being a common songbird in North America, the song sparrow is a valuable model organism for conservation biology, population and evolutionary research: it is a very well studied species, abundant, and inhabiting even small habitat patches with varying density (Nice, 1937; Arcese et al., 2002).

Study system: the song sparrow population of Mandarte Island

The study population is located on a small island, Mandarte Island, which lies in the Gulf Islands in southern British Columbia, and is part of the traditional territory of the Tsawout and Tseycum First Nations bands. The island is ca. 700m long and 85m wide, and multiple small islands, as well as Vancouver Island are within 5 km distance, and also host song sparrows (Smith et al., 2006, Fig. 3).

Mandarte is uninhabited by humans, and experiences a sub-Mediterranean climate regime with mild, wet winters, and warm, dry summers, when fresh water can be absent from the island. The island is covered by a mixture of shrubs and meadows. It also hosts a large sea bird colony consisting of pelagic and double-crested cormorants (*Phalacrocorax pelagicus*; *P. auritus*), pigeon guillemots (*Cepphus columba*), rhinoceros auklets (*Cerorhinca monocerata*), and Glaucous-winged gulls (*Larus glaucescens,* Lameris et al., 2016). Further, recently (~2010), fox sparrows (*Passerella unalaschcensis)* became the most abundant passerine on Mandarte (Visty et al., 2018).

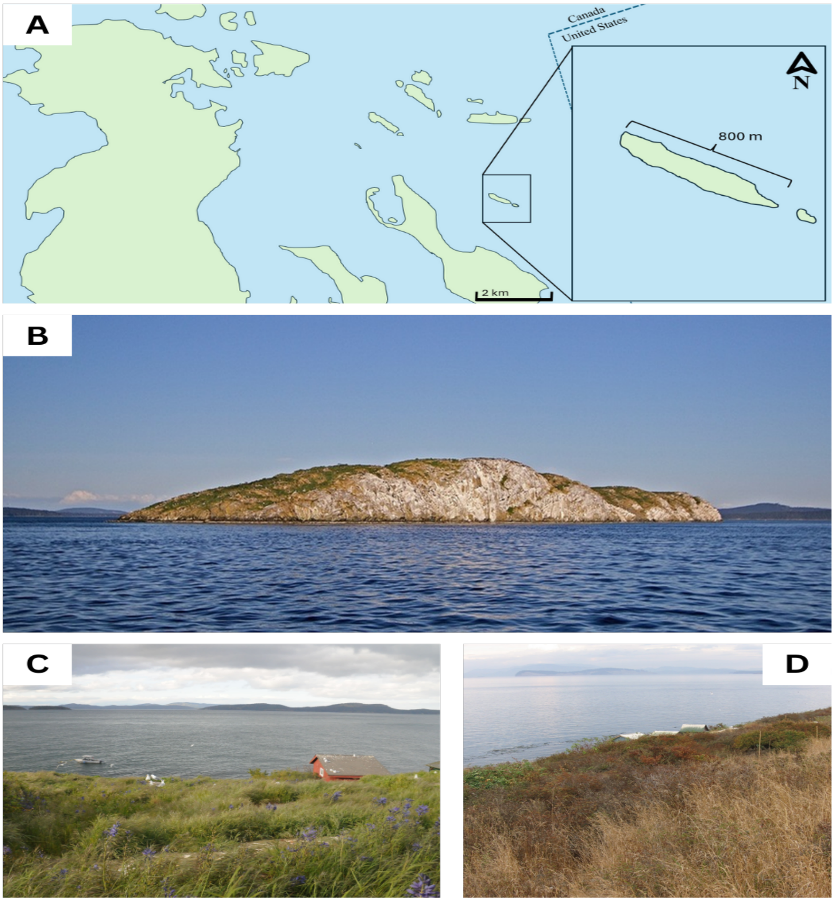


Fig. 3: Map of the study area, showing Mandarte within its surrounding, with Vancouver island and multiple small islands within 5 km radius, and zoomed map showing the size of (A), view of the whole Mandarte Island from the sea (B), and pictures taken on Mandarte Island showing the vegetation in spring (C) and summer (D). Map: Rune Sørås, Photos: J. M. Reid and L. Dickel.

Song sparrows are typically resident on Mandarte, i.e., spend the whole year on the island. While fieldwork on Mandarte was initiated from the 1950s, since 1975, individuals were continuously individually marked and yearly observed, and all territories and nest were mapped every year (Arcese, 1992, Smith et al., 2006). This extensive fieldwork resulted in complete population counts and individual based life history data.

Specifically, each spring, all territories are mapped and individuals which occupy them are observed and identified, as well as non-territorial floaters. Breeding activities are monitored on a weekly basis, such that all nests are found. Thereby, nestlings are banded approximately 6 days after hatching (with one numbered metal band and three plastic color bands, Fig. 4). For any nests which are not found before independence of the young, the fledglings are captured in a mist net at ~20 days of age. Consequently, all individuals hatching locally on Mandarte are banded, which allows individual identification subsequently. This systems allows to identify immigrants as unbanded adults present on Mandarte early in each breeding season. Further, sexing of adults is done by behavioral observation during breeding, as males sing and females incubate the clutch of eggs. These observations allow us to link all nests, and hence all individually identifiable nestlings, to their parents (Fig. 4). A social pedigree has therefore been constructed since 1975 (with complete data, except for a gap due to reduced fieldwork in 1981, Arcese et al., 1992).

Further, since 1993, blood samples were taken from all individuals either when they are still in the nest, or from adults captured in mist nets. This allowed typing at polymorphic microsatellite markers. For all individuals 13 main loci are available, which are sufficient to determine genetic parentage with high certainty. For some years, up to 160 microsatellite markers are available. Hence, the pedigree has now been genetically corrected (Sardell et al., 2010; Reid and Sardell, 2012, Nietlisbach et al., 2017), and it was found that ~28% of chicks are sired through extra-pair paternity (Sardell et al., 2010; Reid et al., 2014). These genetic marker data also allowed the verification of immigrant status (Nietlisbach et al., 2015, paper I), and sex assignment, particularly for individuals which did not survive to adulthood (Postma et al., 2011).

The small, focal population (12 to 159 adults per year between 1976-2018) lies within multiple small, typically sedentary song sparrow populations which are linked by occasional dispersal, so that occasionally immigrants arrive (Smith et al., 1996, 2006; Marr et al., 2002; Wilson and Arcese, 2008; paper I; Reid et al., 2021). After arrival they are captured in mist nets and also individually marked with color bands (Arcese et al., 1992; Keller, 1998; Marr et al., 2002; Reid et al., 2014, Fig. 7 G,H). In total, the population on Mandarte received 48 immigrants between 1976-2016 (30 females, 18 males; mean 1.2/year, range 0-4; paper I). These immigrants made very critical genetic contributions to the population, including the recovery after a bottleneck (Keller et al., 2001; Wolak et al., 2018; Reid and Arcese, 2020; Reid et al., 2021). Currently, the population is again near extinction, but might be rescued through the arrival of female immigrants after the extinction of female lines on the island. Hence, the focal population is part of a metapopulation, where extinction and recolonisation dynamics are likely to occur on a larger time scale (Hanski and Simberloff, 1997).

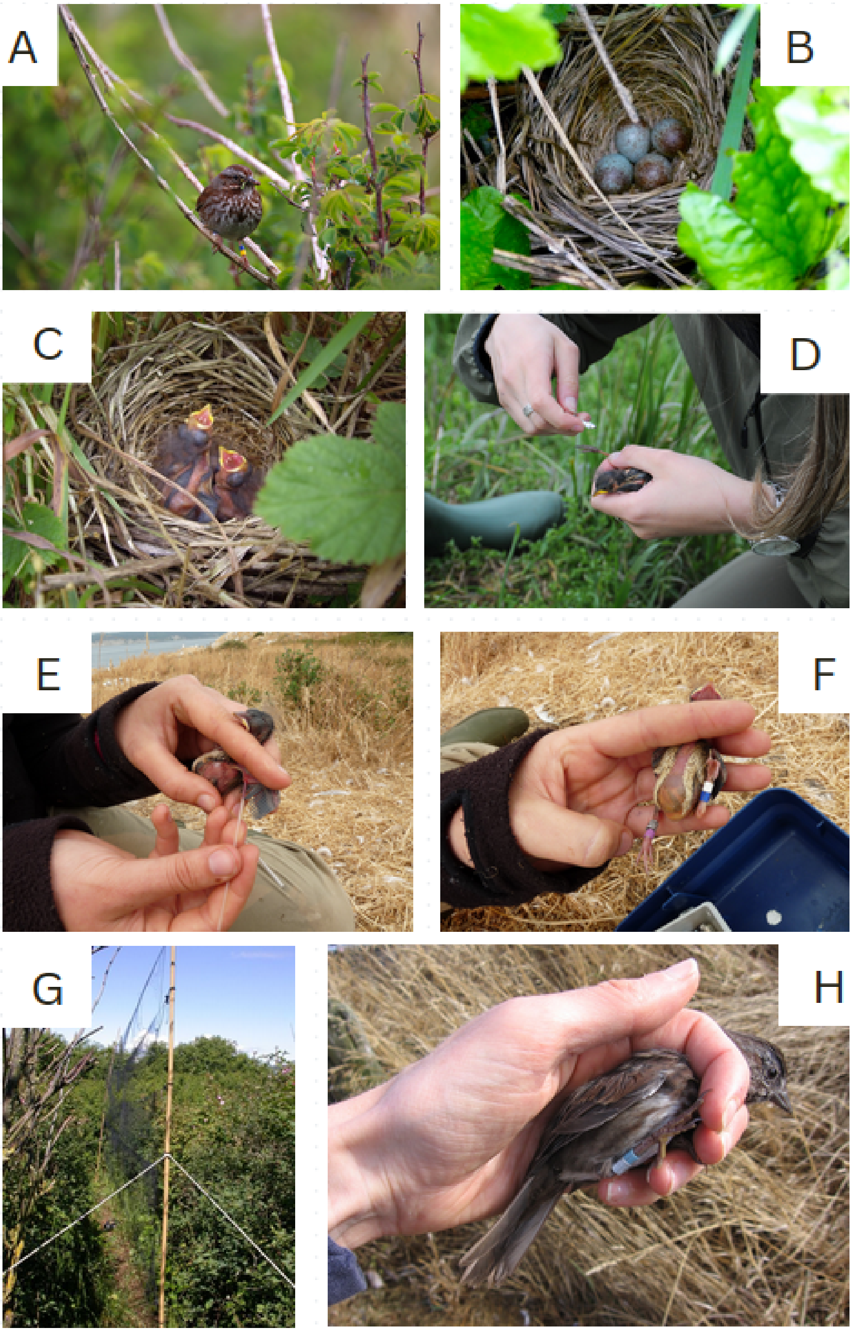

A
B
C
D
E
F
G
H

Fig 4.: Field work aspects of the song sparrow system capturing the key processes of data collection. Banded adult carrying an insect larva (A). Song sparrow nest (B), and chicks (C). Banding of chicks ~6 days of age (D), blood sampling of a chick for genotyping and genetic correction of the pedigree (E), banded nestling (F), mist net to capture adult birds including unbanded immigrants (G), and adult captured bird with color bands for individual identification (H). Photos by J. M. Reid, P. Nietlisbach, R. Germain, and L. Dickel.

Statistical paradigm

In this thesis, I used statistical methods in a flexible way, combining frequentist and Bayesian methods, where they were most useful. Especially for approximating fitness I utilized a Bayesian framework (paper II), this was very useful, as I could estimate fitness components separately and calculate specific post-hoc comparisons for inference. This also allowed me to isolate potentially biased fitness components (specifically juvenile survival), and to compare post-dispersal fitness components of immigrants with locally hatched groups. Further, the flexibility of Bayesian methods allowed creative ways of uncovering „unobservable“ fitness components of immigrants (Supporting information, paper II). These methods also allowed to report full posterior distributions, and consequently to propagate uncertainties fully when combining fitness components.

Through my contribution as co-author to two publications during my PhD, I got further understanding of the importance of quantifying and propagating uncertainty in ecology and evolution (Simmonds et al., 2022; 2023), and tried to realize these insights in my work by giving informative estimates with fully quantified uncertainties.

Paper summaries

Paper I: Are immigrants outbred and unrelated? Testing standard assumptions in a wild metapopulation

Theoretical and empirical work in conservation, evolutionary biology and animal biology rely typically on three assumptions about immigrants; specifically, that they are outbred, unrelated to each other and unrelated to the recipient population at the time of arrival (Ballou, 1983; Hammerly et al., 2016; Pemberton, 2008, Fig. 1). Despite these assumptions being fundamental for these research areas, they are rarely empirically tested in wild populations. I tested these assumptions explicitly for the song sparrows of Mandarte Island.

Specifically, I was able to assess inbreeding and relatedness status of immigrants relative to the local population. This was possible due to the availability of microsatellite marker data for both the local population and immigrants, and detailed pedigree data for the resident population. Hence, I established the relationship between marker homozygosity and pedigree f, and then used observed, and hypothetical homozygosity of offspring as a measure to compare both immigrants and their descendants to natives on the local homozygosity-pedigree f scale.

I found that immigrants were on average outbred relative to the local population, with their homozygosity falling within the predicted range of homozygosity of natives with pedigree f=0. Equally, the offspring and hypothetical offspring between native-immigrant pairings and immigrant-immigrant pairings were heterozygous on the local scale, indicating that immigrants were both unrelated to each other, and to natives.

Hence, I explicitly validated the three standard assumptions of evolutionary biology for a key study system of evolutionary biology.

This study set the scene for formulating and interpreting fitness effects of immigrants into this population (paper II, paper III). Therefore, I set up predictions for the following work accordingly, specifically that immigrants would likely increase local genetic variation and potentially induce heterosis by causing outbreeding in their offspring (Charlesworth and Willis, 2009).

Paper II: Contrasting multi-generational fitness effects of natural immigration indicate strong heterosis and epistatic breakdown in a wild bird population

Following the results of paper I, that immigrants are unrelated to natives, I expected genetic and fitness effects following immigration. Hence, I tested for heterosis and epistatic breakdown, using line-cross theory and specific planned comparisons among immigrants, natives and their F1, F2 and backcross descendants (Fig. 2). I estimated multiple fitness components separately for males and females of all these groups, specifically, egg-to-banding survival probability, juvenile survival probability, and lifetime reproductive success. Then, I conducted specific post-hoc comparisons in order to infer genetic effects underlying fitness differences between the groups and approximated overall fitnesses (Fig. 1).

Thereby, I discovered striking and unexpected fitness differences among different filial groups. Specifically, I found strong heterosis in offspring of native-immigrant matings (i.e. F1s) and severe epistatic breakdown in F1-F1 offspring (i.e. F2s, Fig. 1). In these effects there were differences between males and females, and among the different fitness components. Especially strong heterosis was apparent in zygote survival and adult male lifetime reproductive success. Epistatic breakdown was particularly strong in male annual and lifetime reproductive success, and in female survival.

Because I included immigrants from diverse sources, the results highlight that heterosis and epistatic breakdown are likely occurring across diverse environments and populations. Hence, I show empirically that immigration can have surprisingly strong fitness effects in natural populations even in the absence of severe local inbreeding.

Paper III: Multi-generational genetic contributions of immigrants reveal cryptic elevated and sex-biased effective gene flow within a natural meta-population

The complex and conflicting fitness effects observed in immigrants’ descendants across two generations led to the question of whether immigrants’ genetic contributions to the local population would persist over longer time frames. Immigration might result in disproportionate effective gene flow compared to the observed number of immigrants given they induce both heterosis and epistatic breakdown.

Hence, collaborators and I used the long-term pedigree data to track expected genetic contributions of immigrants and natives for up to 15 years following arrival and recruitment, including all individuals for which all descendants have been genetically verified. We used gene-drop simulations to estimate for each cohort the number of copies of a hypothetical allele stemming from any of the initially present natives or immigrants in the subsequent cohorts.

We found that consistent with heterosis-enhanced introgression, genetic contributions of immigrants exceeded those of natives. We also detected a strong sex by status (immigrant or native) interaction, whereby female immigrants’ genetic contributions exceeded those of natives up to 3-4 times, while male lineages typically went extinct.

We show complex multi-generational trajectories of introgression and extinction of lineages matching the theoretical predictions that heterosis can increase gene flow, i.e. lead to effectively higher immigration rates than initially observed (Ingvarsson and Whitlock, 2000). Based on the results of paper II, we expected that effective rates of gene flow could be higher than expected from observed immigration rates due to heterosis, or that they could be low because of low fitness in the F2s. Indeed, there was abundant introgression, more than expected from the numbers of arriving immigrants. However, surprisingly this introgression resulted primarily from female immigrant lineages.

Paper IV: Does non-random mating modulate the multi-generational fitness effects of immigration?

Given the observed fitness patterns (paper II), demographic effects of immigrants depend beside the magnitude of fitness consequences also on the frequency with which specific crosses with different fitnesses are produced. From this, multiple questions arose. First, whether behavioral mechanisms were contributing to patterns of heterosis and epistatic breakdown in reproductive success (paper II). Second, given low frequencies of F2 individuals in the population and their low fitness, I asked whether F1 individuals were pairing less than expected with each other, thereby preventing the production of low fitness F2 descendants.

Hence, to test for heterosis and epistatic breakdown in social pairing success I quantified pairing probabilities for immigrants, natives and their descendant groups. Then, to test for non-random pairing, I compared observed pairing frequencies against two biologically meaningful null models.

I found patterns of low immigrant success and heterosis in social pairing success of male immigrants and F1s individuals respectively. Hence, the ability of acquiring a social partner underpins and thereby contributes to the patterns of lifetime reproductive success (paper II). However, such patterns were not evident for females, which show little variation in their social pairing success. I found no evidence for avoidance among F1 individuals, and hence no evidence for mechanisms preventing the production of low fitness F2 offspring. Hence, I show that pairing success is a mechanism contributing to sex-specific fitness and introgression, but not through any substantive modulation of pairing frequencies. These results highlight the complexity and multitude of sex-specific mechanisms underpinning introgression.

Discussion & Future Directions

In this thesis, I contributed to the understanding of genetic, fitness and evolutionary consequences of immigration in a natural metapopulation. I tested standard assumptions of evolutionary biology in a small population of song sparrows, showing that immigrants were indeed unrelated to the recipient population. Then, I showed resulting striking sex-specific fitness and introgression patterns following immigration, and highlighted social pairings as one underlying mechanism.

This work highlights the complexity of the effects of immigration even in systems which are not largely diverged or experiencing severe inbreeding. Strong and multifaceted non-additive genetic effects of immigration within natural metapopulation systems receiving regular immigrants are possible, including both heterosis and epistatic breakdown. This result might seem to be contradicting some of previous genetic rescue work, where epistatic breakdown (and resulting outbreeding depression) is suggested to be minor (Frankham et al, 2011; Ralls et al., 2018). Conversely, my results are consistent with genetic incompatibility following recombination among native and immigrant genomes which were mainly investigated in inbred lines or inter-species context (Mather and Jinks, 1977; Lynch, 1991; Lynch and Walsh, 1998). Thereby I show novel empirical estimates for effects of immigration in a natural population not experiencing severe inbreeding, thus relating to theoretical predictions of heterosis-enhanced introgression in weakly subdivided populations (Ingvarsson and Whitlock, 2000; Whitlock et al., 2000; Edmands, 2007; Charlsworth, 2018).

Obstacles for studying fitness effects of immigration are lack of comprehensive pedigrees for wild populations (Pemberton, 2008) or metapopulations, detailed knowledge of immigration, and fitness components for immigrant individuals, dependent on the age and stage of dispersal of a species (Furrer and Pasinelli, 2016). More interest in the fitness effects of immigration in both naturally and anthropogenically fragmented populations has emerged (Furrer and Pasinelli 2016; Millon et al., 2019), and genetic marker data allow more readily construction of pedigrees in the wild (Pemberton, 2008). Simultaneous consideration of positive and negative fitness effects of immigration now become more common (e.g. in a meta-analysis of between-population outbreeding of wild populations, Whitlock et al., 2013; genetic management of small and inbred populations reviewed in Liddell et al., 2021). Further, constructing pedigrees in wild populations is now more feasible and accessible due to availability of genetic and genomic marker data (Pemberton, 2008; Galla et al., 2022). The use of genomic methods could offer even more insight into the specific history of individual inbreeding events (Kardos et al., 2015; Niskanen et al., 2020), tracking the fate of specific immigrant genes throughout generations, and uncover the specific regions and genetic architecture of local adaptation (e.g. Walsh et al., 2019). Such data are already available for some systems (e.g. Atlantic salmon, *Salmo salar,* Mobley et al., 2019; Florida Scub-Jay *Aphelocoma coerulescens,* Chen et al., 2019; North American red squirrel *Tamiasciurus hudsonicus,* Martinig et al., 2020; Tinidadian guppy, *Poecilia reticulata,* Fitzpatrick et al., 2020; house sparrow *Passer domesticus*, Niskanen et al., 2020). While estimates of fitness components from immigrant crosses and descendants are not yet widely available, I hope that the conceptual advances and interesting results of this work will contribute to stimulate similar work across various systems.

Expanding such methods to whole metapopulation systems, or including multiple source and recipient population, would be a valuable next advance with the possibility of combined study of both emigration and immigration. Pedigrees encompassing whole metapopulation systems are rarely available, including knowledge of the specific environment of origin of the immigrants (but see house sparrow *Passer domesticus*, Billing et al., 2012; Niskanen et al., 2020). However, applying methods developed and used in this thesis to systems where pedigrees and fitness are known for whole metapopulations could further advance the ability to relate patterns of genetic architecture of fitness across environmental conditions and thereby understand fine scale local adaptation processes.

Likely, immigrant effects are frequently complex and multifaceted, entailing trade-offs between costs and benefits, which then cumulate in net effects on population dynamics and persistence. Availability of estimates quantifying these complex effects across different taxa and population parameters will then help to inform predictions about both natural and facilitated immigration across different systems. Then, these results can add novel insight for the theory and application of genetic rescue, and in particular open the possibility for the simultaneous occurrence of positive and negative fitness effects of immigration.

Further availability of such detailed empirical estimates across multiple populations and metapopulations can then meet methodological and computational advances, to bring forward both evolutionary biology and conservation and management. When multiple parameters are estimated for a population, simulations and counter-factual thinking can be applied to gain additional insights. Using such methodologies, hypothetical outcomes of increased or decreased immigration can be simulated and can then directly inform management of endangered populations where population parameters are known. Then, the net impact of immigrants impacting the persistence or extinction probability of a population can be estimated. Thereby, counterfactuals can support us to imagine what would have happened if immigrants had, in fact, not arrived in a population, or if the number of immigrants would have differed from the observed number (Coetzee and Gaston, 2021; McMurdo Hamilton et al., 2023).

This work highlights some of the complexity of fitness and genetic consequences of immigration within a wild metapopulations, and how connecting aspects of different research areas offers both exciting questions, and insights into the complex causes and consequences of animals’ life histories. Such complex and sometimes conflicting effects following immigration make reconciling and collaborating across different fields, such as speciation and hybridization research, evolutionary, conservation, and behavioral biology an important and to be continued effort.

Literature cited

Åkesson, M., Ø. Flagstad, J. Aspi, I. Kojola, O. Liberg, P. Wabakken, and H. Sand. 2022. Genetic signature of immigrants and their effect on genetic diversity in the recently established scandinavian wolf population. Conservation Genetics, 23: 359–73.

Åkesson, M., O. Liberg, H. Sand, P. Wabakken, S. Bensch, and Ø. Flagstad. 2016. Genetic rescue in a severely inbred wolf population. Molecular Ecology, 25: 4745–56.

Allendorf, F. W. , R. F. Leary, N. P. Hitt, K. L. Knudsen, L. L. Lundquist, and P. Spruell. 2004. Intercrosses and the U.S. endangered species act: should hybridized populations be included as westslope cutthroat trout? Conservation Biology, 18: 1203-1212.

Andersson, M. 1994. Sexual Selection. Princeton University Press.

Arcese, P., J. N. M. Smith, and W. M. Hochachka. 1992. Stability, regulation, and the determination of abundance in an insular song sparrow population. Ecological Society of America, 73: 805–822.

Arcese, P., M. K. Sogge, A. B. Marr, and M. A. Patten. 2002. Song Sparrow (*Melospiza melodia*). The birds of North America. Inc., 2002.

Atsumi, K., M. Lagisz, and S. Nakagawa. 2021. Nonadditive genetic effects induce novel phenotypic distributions in male mating traits of F1 hybrids. Evolution 75: 1304–15.

Ballou, J. 1983. Calculating inbreeding coefficients from pedigrees. In C. M. Schoenwald-Cox, S. M. Chambers, B. MacBride, and L. Thomas (Eds.), Genetics and conservation: A reference for managing wild animal and plant populations (pp. 509–516). Benjamin Cummings.

Barbraud, C. and K. Delord. 2020. Selection against immigrants in wild seabird populations. Ecology Letters, 24: 84-93.

Bell, G. 2013. Evolutionary rescue and the limits of adaptation. Philosophical Transactions of the Royal Society B, 368: 20120080.

Bell, D. A., Z. L. Robinson, W. C. Funk, S. W. Fitzpatrick, F. W. Allendorf, D. A. Tallmon, and A. R. Whiteley. 2019. The exciting potential and remaining uncertainties of genetic resuce. Trends in Ecology and Evolution, 34:1070-1079.

Billing, A. M., A. M. Lee, S. Skjelseth, Å. A. Borg, M. C. Hale, J. Slate, H. Pärn, T. H. Ringsby, B. E. Sæther, and H. Jensen. 2012. Evidence of inbreeding depression but not inbreeding avoidance in a natural house sparrow population. Molecular Ecology, 21: 1487–99.

Bolnick, D. I., and P. Nosil. 2007. Natural selection in populations subject to a migration load. Evolution, 61: 2229–2243.

Carlson, S. M, Cunningham, C. J., and Westley, P. A. H. 2014. Evolutionary rescue in a changing world. Trends in Ecology & Evolution, 29:521-530.

Charlesworth, B. 2018. Mutational load, inbreeding depression and heterosis in subdivided populations. Molecular Ecology, 27: 4991-5003.

Charlesworth, D., and J. H. Willis. 2009. The genetics of inbreeding depression. Nature Reviews Genetics, 10: 783–96.

Chan Y., and P. Arcese. 2003. Morphological and microsatellite differentiation in *Melospiza melodia* (Aves) at a microgeographic scale. Journal of Evolutionary Biology, 16:939–947.

Chen, N., I. Juric, E. J. Cosgrove, R. Bowman, J. W. Fitzpatrick; S. J. Schoech, A. G. Clark, and G. Coop. Allele frequency dynamics in a pedigreed natural population. Proceedings of the National Academy of Sciences, 116: 2158-2164.

Coetzee, B. W. T. and K. J. Gaston. 2021. An appeal for more rigorous use of counterfactual thinking in biological conservation. Conservation Science and Practice, 3: e409.

Crnokrak, P., and D. A. Roff. 1999. Inbreeding depression in the wild. Heredity, 83: 260–70.

Derry, A. M., D. J. Fraser, S. P. Brady, L. Astorg, E. R. Lawrence, G. K. Martin, J.-M. Matte, et al. 2019. Conservation through the lens of (mal)adaptation: concepts and meta-analysis. Evolutionary Applications, 12: 1287–1304.

Doligez, B., and T. Pärt. 2008. Estimating fitness consequences of dispersal: A road to ‘know-where’? Non-random dispersal and the underestimation of dispersers’ fitness. Journal of Animal Ecology, 77: 1199–1211.

Edelaar, P., and D. I. Bolnick. 2012. Non-random gene flow: An underappreciated force in evolution and ecology. Trends in Ecology and Evolution, 27: 659–665.

Edelaar, P. and D. I. Bolnick, 2019. Appreciating the multiple processes increasing individual or population fitness. Trends in Ecology & Evolution, 34: 435–46.

Edmands, S. 2007. Between a rock and a hard place: evaluating the relative risks in inbreeding and outbreeding for conservation and management. 16: 463-475.

Epinat, G., and T. Lenormand. 2009. The evolution of assortative mating and selfing with in- and outbreeding depression. Evolution, 63: 2047–60.

Fenster, C. B., and L. F. Galloway. 2000. Population differentiation in an annual legume: genetic architecture. Evolution, 54: 1157–72.

Fitzpatrick, S. W., G. S. Bradburd, C. T. Kremer, P. E. Salerno, L. M. Angeloni, and W. C. Funk. 2020. Genomic and fitness consequences of genetic rescue in wild populations. Current Biology, 30: 517-522.e5.

Fitzpatrick, S. W., J. C. Gerberich, L. M. Angeloni, L. L. Bailey, E. D. Broder, J. Torres-Dowdall, C. A. Handelsman, et al. 2016. Gene flow from an adaptively divergent source causes rescue through genetic and demographic factors in two wild populations of Trinidadian guppies. Evolutionary Applications, 9:879–891.

Fitzpatrick, S. W, C. Mittan-Moreau, M. Miller, and J. M. Judson. 2023. Genetic rescue remains underused for aiding recovery of federally listed vertebrates in the United States. Journal of Heredity, 114: 354–66.

Fitzpatrick, S. W., and B. N. Reid. 2019. Does gene flow aggravate or alleviate maladaptation to environmental stress in small populations? Evolutionary Applications, 12: 1402–16.

Frankham, R. 1998. Inbreeding and extinction: Island Populations. Conservation Biology 12: 665-675.

Frankham, R. 2010. Challenges and opportunities of genetic approaches to biological conservation. Biological Conservation, 143: 1919-1927.

Frankham, R., J. D. Ballou, M. D. B. Eldridge, R. C. Lacy, K. Ralls, M. R. Dudash, and C. B. Fenster. 2011. Predicting the probability of outbreeding depression. Conservation Biology, 25: 465–475.

Furrer, R. D. And G. Pasinelli. 2016. Empirical evidence for source-sink populations: a review on occurrence, assessment and implications. Biological Reviews, 91: 782-795.

Galla, S. J., L. Brown, Y. Couch-Lewis, I. Cubrinovska, D. Eason, R. M. Gooley, J. A. Hamilton et al. 2022. The relevance of pedigrees in the conservation genomics era. Molecular Ecology, 31: 41-54.

Garant, D., Forde, S. E., and Hendry, A. P. 2007. The multifarious effects of dispersal and gene flow on contemporary adaptation. Functional Ecology, 21: 434-443.

Goedert, D., H. Jensen, L. Dickel and J. M. Reid. *Unpublished manuscript.* Multi-generational fitness legacies of natural immigration: theoretical and empirical perspectives and opportunities.

Gomulkiewicz, R., and R. G. Shaw. 2013. Evolutionary rescue beyond the models. Philosophical Transactions of the Royal Society B, 368: 20120093.

Haddad, N. M., L. A. Brudvig, J. Clobert, K. F. Davies, A. Gonzalez, R. D. Holt, T. E. Lovejoy, et al. 2015. Habitat fragmentation and its lasting impact on earth’s ecosystems. Science Advances, 1: 1–10.

Haila, Y. 2002. A Conceptual genealogy of fragmentation research: From island biogeography to landscape ecology. Ecological Applications, 12: 321–34.

Hammerly, S. C., D. A. de la Cerda, H. Bailey, and J. A. Johnson. 2016. A pedigree gone bad: increased offspring survival after using DNA-based relatedness to minimize inbreeding in a captive population. Animal Conservation, 19: 296–303.

Hanski, I. And Simberloff, D. The metapopulation approach, its history, conceptual domain, and application to conservation. Chapter in: Hanski, I and M. Gilpin. Metapopulation Biology: Ecology, Genetics, and Evolution. 1997. San Diego, CA

Hoffmann, A. A., A. D. Miller, & A. R. Weeks. 2021. Genetic mixing for population management: From genetic rescue to provenancing. Evolutionary Applications, 14: 634-652.

Hufbauer, R. A., M. Szűcs, E. Kasyon, C. Youngberg, M. J. Koontz, C. Richards, T. Tuff, and B. A. Melbourne. 2015. Three types of rescue can avert extinction in a changing environment. Proceedings of the National Academy of Sciences, 112: 10557–10562.

Ingvarsson, P. K., and M. C. Whitlock. 2000. Heterosis increases the effective migration rate. Proceedings of the Royal Society B, 267: 1321–26.

Jiang, Y., D. I. Bolnick, and M. Kirkpatrick. 2013. Assortative mating in animals. The American Naturalist, 181: E125-E138.

Kardos, M., G. Luikart, and F. W. Allendorf. 2015. Measuring individual inbreeding in the age of genomics: marker-based measures are better than pedigrees. Heredity, 115: 63–72.

Kardos, M., M. Åkesson, T. Fountain, Ø. Flagstad, O. Liberg, P. Olason, H. Sand, P. Wabakken, C. Wikenros, and H. Ellegren. 2018. Genomic consequences of intensive inbreeding in an isolated wolf population. Nature Ecology and Evolution, 2: 124–31.

Kawecki, T. J. and D. Ebert. 2004. Conceptual issues in local adaptation. Ecological Letters, 7: 1225-1241.

Keller, L. F. 1998. Inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). Evolution, 52: 240–50.

Keller, L. F., and P. Arcese. 1998. No evidence for inbreeding avoidance in a natural population of song sparrows (*Melospiza melodia*). The American Naturalist, 152: 380–392.

Keller, L. F., K. J. Jeffery, P. Arcese, M. A. Beaumont, W. M. Hochachka, J. N. Smith, and M. W. Bruford. 2001. Immigration and the ephemerality of a natural population bottleneck: Evidence from molecular markers. Proceedings of the Royal Society B, 268: 1387–94.

Keller, L. F., J. M. Reid, and P. Arcese. 2008. Testing evolutionary models of senescence in a natural population: age and inbreeding effects on fitness components in song sparrows. Proceedings of the Royal Society B, 275: 597–604.

Keller, L. F., and D. M. Waller. 2002. Inbreeding effects in wild populations. Trends in Ecology and Evolution, 17: 230–41.

Lameris, T. K., Bennett, J. R., Blight, L. K., Giesen, M., Janssen, M. H., Schaminée, J.J.H.J, P. Arcese. 2016. A century of ecosystem change: human and seabird impacts on plant species extirpation and invasion on islands. PeerJ, 4: e2208.

Lamichhaney, S., F. Han, M. T. Webster, L. Andersson, B. R. Grant, and P. R. Grant. 2018. Rapid hybrid speciation in Darwin’s finches. Science, 359: 224–28.

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

Li, X.-Y., and H. Kokko. 2019. Intersexual resource competition and the evolution of sex-biased dispersal. Frontiers in Ecology and Evolution, 7:111.

Liddell, E., P. Sunnucks, and C. N. Cook. 2021. To mix or not to mix gene pools for threatened species management? Few studies use genetic data to examine the risks of both actions, but failing to do so leads disproportionately to recommendations for separate management. Biological Conservation, 256: 109072.

Lynch, M. 1991. The genetic interpretation of inbreeding depression and outbreeding depression. Evolution, 45: 622-629.

Lynch, M., and B. Walsh. 1998. Genetics and analyses of quantitative traits. Sinauer Associates, Inc., Sunderland.

MacArthur, R. H., and E. O. Wilson. 1967. The theory of island biogeography. Princeton, Princeton University Press.

Marr, A. B., L. F. Keller, and P. Arcese. 2002. Heterosis and outbreeding depression in descendants of natural immigrants to an inbred population of song sparrows (*Melospiza melodia*). Evolution, 56: 131–42.

Marshall, J. A., 1948. Ecologic races of the song sparrow in the San Francisco Bay region. I. Habitat and abundance. Condor, 50:193-215.

Martinig, A. R., A. G. McAdam, B. Dantzer, J. E. Lane, D. W. Coltman, and S. Boutin. 2020. The new kid on the block: immigrant males win big whereas females pay fitness cost after dispersal. Ecology Letters, 23: 430–38.

Mather, K. and J. L. Jinks. 1977. Introduction to biometrical genetics. Chapman & Hall, London.

McMurdo Hamilton, T., J. G. Ewen, A. J. Beauchamp, T. Makan, M. Rowcliffe and S. Canessa. 2023. Data-driven counterfactual evaluation of management outcomes to improve emergency conservation decisions. Conservation Letters, 16: e12925.

Millon, A., X. Lambin, S. Devillard, and M. Schaub. 2019. Quantifying the contribution of immigration to population dynamics: A review of methods, evidence and perspectives in birds and mammals. Biological Reviews, 94: 2049–2067.

Mobley, K. B., H. Granroth-Wilding, M. Ellmen, J. P. Vähä, T. Aykanat, S. E. Johnston, P. Orell, J. Erkinaro, and C. R. Primmer. 2019. Home ground advantage: Local atlantic salmon have higher reproductive fitness than dispersers in the wild. Science Advances, 5: 1–9.

Muraro, M., M. Falaschi, and G. F. Ficetola. 2022. Patterns of performance variation between animal hybrids and their parents: A meta-analysis. Evolutionary Biology, 49: 482–96.

Nietlisbach, P., G. Camenisch, T. Bucher, J. Slate, L. F. Keller, and E. Postma. 2015. A microsatellite-based linkage map for song sparrows (*Melospiza melodia*). Molecular Ecology Resources, 15: 1486–1496.

Nietlisbach, P., L. F. Keller, G. Camenisch, F. Guillaume, P. Arcese, J. M. Reid, and E. Postma. 2017. Pedigree-based inbreeding coefficient explains more variation in fitness than heterozygosity at 160 microsatellites in a wild bird population. Proceedings of the Royal Society B: Biological Sciences, 284: 1850.

Nice, M. M. 1937. Studies in the life history of song sparrows. I. Transactions of the Linnean Society of New York, 4:1-273.

Niskanen, A. K., A. M. Billing, H. Holand, I. J. Hagen, Y. G. Araya-Ajoy, A. Husby, B. Rønning, A. M. Myhre, ... and H. Jensen. 2020. Consistent scaling of inbreeding depression in space and time in a house sparrow metapopulation. Proceedings of the National Academy of Sciences of the United States of America, 117: 14584–14592.

Orr, H. A. 1995. The population genetics of speciation: The evolution of hybrid incompatibilities. Genetics, 139: 1805–1813.

Patten, M. A., J. T. Rotenberry, and M. Zuk. 2004. Habitat selection, acoustic adaptation, and the evolution of reproductive isolation. Evolution 58: 2144-2155.

Pärssinen, V., K. Hulthén, C. Brönmark, C. Skov, J. Brodersen, H. Baktoft, B. B. Chapman, L.-A. Hansson, and P. A. Nilsson. 2020. Maladaptive migration behaviour in hybrids links to predator-mediated ecological selection. Journal of Animal Ecology, 89: 2596–2604.

Pemberton, J. M. 2008. Wild pedigrees: the way forward. Proceedings of the Royal Society B, 275: 613–621.

Pickup, M., Y. Brandvain, C. Fraïsse, S. Yakimowski, N. H. Barton, T. Dixit, C. Lexer, E. Cereghetti, and D. L. Field. 2019. Mating system variation in hybrid zones: Facilitation, barriers and asymmetries to gene flow. The New Phytologist, 224: 1035–1047.

Postma, E., F. Heinrich, U. Koller, R. J. Sardell, J. M. Reid, P. Arcese, and L. F. Keller. 2011. Disentangling the effect of genes, the environment and chance on sex ratio variation in a wild bird population. Proceedings of the Royal Society B, 278:2996-3002.

Ralls, K., J. D. Ballou, M. R. Dudash, M. D. B. Eldridge, C. B. Fenster, R. C. Lacy, P. Sunnucks, and R. Frankham. 2018. Call for a paradigm shift in the genetic management of fragmented populations. Conservation Letters, 11: 1–6.

Reid, J. M., and P. Arcese. 2020. Recent immigrants alter the quantitative genetic architecture of paternity in song sparrows. Evolution Letters, 4: 124–136.

Reid, J. M., P. Arcese, G. Bocedi, A. B. Duthie, M. E. Wolak, and L. F. Keller. 2015. Resolving the conundrum of inbreeding depression but no inbreeding avoidance: Estimating sex-specific selection on inbreeding by Song Sparrows (*Melospiza melodia*). Evolution, 69: 2846–2861.

Reid, J. M., P. Arcese, P. Nietlisbach, and M. E. Wolak. 2021. Immigration counter-acts local micro-evolution of a major fitness component: Migration-selection balance in free-living song sparrows. Evolution Letters, 5:48-60.

Reid, J. M., P. Arcese, R. J. Sardell, and L. F. Keller. 2011. Additive genetic variance, heritability, and inbreeding depression in male extra-pair reproductive success. The American Naturalist, 177: 177–187.

Reid, J. M., G. Bocedi, P. Nietlisbach, A. B. Duthie, M. E. Wolak, E. A. Gow, and P. Arcese. 2016. Variation in parent–offspring kinship in socially monogamous systems with extra-pair reproduction and inbreeding. Evolution, 70: 1512–1529.

Reid, J. M., L. F. Keller, A. B. Marr, P. Nietlisbach, R. J. Sardell, and P. Arcese. 2014. Pedigree error due to extra-pair reproduction substantially biases estimates of inbreeding depression. Evolution, 68: 802–815.

Reid, J. M., and R. J. Sardell. 2012. Indirect selection on female extra-pair reproduction? Comparing the additive genetic value of maternal half-sib extra-pair and within-pair Offspring. Proceedings of the Royal Society B, 279: 1700–1708.

Rhymer, J. M. and D. Simberloff. 1996. Extinction by hybridization and introgression. Annual Review of Ecology and Systematics, 27: 83-109.

Saastamoinen, M., B. Bocedi, J. Cote, D. Legrand, F. Guillaume, C. W. Wheat, E. A. Fronhofer, C. Garcia, R., et al. 2017. Genetics of dispersal. Biological Reviews. 93: 574-599.

Sardell, R. J., L. F. Keller, P. Arcese, T. Bucher, and J. M. Reid. 2010. Comprehensive paternity assignment: Genotype, spatial location and social status in song sparrows, *Melospiza melodia*. Molecular Ecology, 19: 4352–4364.

Smith, J. N. M., M. J. Taitt, C. M. Rogers, P. Arcese, L. F. Keller, A. L. E. V. Cassidy and W. Hochaka. 1996. A metapopulation approach to the population biology of the song sparrow *Melospiza melodia.* Ibis, 138: 120-128.

Smith, J. N. M., L. F. Keller, A. B. Marr, and P. Arcese. 2006. Conservation and biology of small populations: the song sparrows of Mandarte Island. Oxford University Press, Oxford.

Schmidt, E. M., and K. S. Pfennig. 2016. Hybrid female mate choice as a species isolating mechanism: Environment matters. Journal of Evolutionary Biology 29: 865–69.

Simmonds, E. G., K. P. Adjei, B. Cretois, L. Dickel, R. González-Gil, J. H. Laverick, C. P. Mandeville, et al. 2023. Recommendations for quantitative uncertainty consideration in ecology and evolution. Trends in Ecology & Evolution, 28:S0169.

Simmonds, E. G., K. P. Adjei, C. W. Andersen, J. C. H. Aspheim, C. Battistin, N. Bulso, H. M. Christensen, ... L. Dickel, ... and R. B. O'Hara 2022. Insights into the quantification and reporting of model-related uncertainty across different disciplines. Iscience, 25: 105512.

Svensson, E. I., D. Goedert, M. A. Gómez-Llano, F. Spagopoulou, A. Nava-Bolaños, and I. Booksmythe. 2018. Sex differences in local adaptation: What can we learn from reciprocal transplant experiments? Philosophical Transactions of the Royal Society B, 373: 20170420.

Svensson, O., J. Gräns, M. C. Celander, J. Havenhand, E. H. Leder, K. Lindström, S. Schöld, C. van Oosterhout, and C. Kvarnemo. 2017. Immigrant reproductive dysfunction facilitates ecological speciation. Evolution, 71: 2510–21.

Szulkin, M., and B. C. Sheldon. 2008. Dispersal as a means of inbreeding avoidance in a wild bird population. Proceedings of the Royal Society B, 275: 703–711.

Szulkin, M., K. V. Stopher, J. M. Pemberton, J. M. Reid. 2013. Inbreeding avoidance, tolerance, or preference in animals? Trends in Ecology & Evolution, 28: 205-211.

Tallmon, D. A., G. Luikart, R. S. Waples. 2004. The alluring simplicity and complex reality of genetic rescue. Trends in Ecology and Evolution, 19: 489-496.

Visty, H., S. Wilson, R. Germain, J. Krippel, and P. Arcese. 2017. Demography of Sooty Fox Sparrows (*Passerella unalaschcensis*) following a shift from a migratory to resident life history. Canadian Journal of Zoology, 96: 436-440.

Walsh, J., P. M. Benham, P. E. Deane-Coe, P. Arcese, B. G. Butcher, Y. L. Chan, Z. A. Cheviron.... And I. J. Lovette. 2019. Genomics of rapid ecological divergence and parallel adaptation in four tidal marsh sparrows. Evolution Letters, 3: 324-338.

Whitlock, M. C., P. K. Ingvarsson, and T. Hatfield. 2000. Local drift load and the heterosis of interconnected populations. Heredity, 84: 452–57.

Whitlock, R., G. B. Stewart, S. J. Goodman, S. B. Piertney, R. K. Butlin, A. S. Pullin, and T. Burke. 2013. A systematic review of phenotypic responses to between-population outbreeding. Environmental Evidence, 2: 13.

Wilson A. G., Arcese P. 2008. Influential factors for natal dispersal in an avian island metapopulation. Journal of Avian Biology, 36:341–347

Wolak, M. E., P. Arcese, L. F. Keller, P. Nietlisbach, and J. M. Reid. 2018. Sex-specific additive genetic variances and correlations for fitness in a song sparrow (*Melospiza melodia*) population subject to natural immigration and inbreeding. Evolution, 72: 2057–2075.

Drawings of song sparrows on the title page and paper dividers in this thesis were created using AI image generating tools (“Magic Media“ by Canva, and hotpot.ai).

Paper I

Received: 27 May 2021 | Revised: 19 August 2021 | Accepted: 23 August 2021

DOI: 10.1111/mec.16173

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Are immigrants outbred and unrelated? Testing standard assumptions in a wild metapopulation

Lisa Dickel[1] | Peter Arcese[2] | Pirmin Nietlisbach[3] | Lukas F. Keller[4,5] | Henrik Jensen[1] | Jane M. Reid[1,6]

[1]Department of Biology, Centre for Biodiversity Dynamics, Norwegian University of Science and Technology, Trondheim, Norway

[2]Department of Forest & Conservation Sciences, University of British Columbia, Vancouver, British Columbia, Canada

[3]School of Biological Sciences, Illinois State University, Normal, Illinois, USA

[4]Department of Evolutionary Biology & Environmental Studies, University of Zurich, Zurich, Switzerland

[5]Zoological Museum, University of Zurich, Zurich, Switzerland

[6]School of Biological Sciences, University of Aberdeen, Aberdeen, UK

Correspondence
Lisa Dickel, Department of Biology, Centre for Biodiversity Dynamics, Norwegian University of Science and Technology, Trondheim, Norway.
Email: lisa.dickel@ntnu.no

Funding information
Norges Forskningsråd, Grant/Award Number: 223257

Abstract

Immigration into small recipient populations is expected to alleviate inbreeding and increase genetic variation, and hence facilitate population persistence through genetic and/or evolutionary rescue. Such expectations depend on three standard assumptions: that immigrants are outbred, unrelated to existing natives at arrival, and unrelated to each other. These assumptions are rarely explicitly verified, including in key field systems in evolutionary ecology. Yet, they could be violated due to non-random or repeated immigration from adjacent small populations. We combined molecular genetic marker data for 150–160 microsatellite loci with comprehensive pedigree data to test the three assumptions for a song sparrow (*Melospiza melodia*) population that is a model system for quantifying effects of inbreeding and immigration in the wild. Immigrants were less homozygous than existing natives on average, with mean homozygosity that closely resembled outbred natives. Immigrants can therefore be considered outbred on the focal population scale. Comparisons of homozygosity of real or hypothetical offspring of immigrant-native, native-native and immigrant-immigrant pairings implied that immigrants were typically unrelated to existing natives and to each other. Indeed, immigrants' offspring would be even less homozygous than outbred individuals on the focal population scale. The three standard assumptions of population genetic and evolutionary theory were consequently largely validated. Yet, our analyses revealed some deviations that should be accounted for in future analyses of heterosis and inbreeding depression, implying that the three assumptions should be verified in other systems to probe patterns of non-random or repeated dispersal and facilitate precise and unbiased estimation of key evolutionary parameters.

KEYWORDS
conservation genetics, gene flow, homozygosity, migration, pedigree inbreeding, relatedness

---

1 | INTRODUCTION

Immigration and resulting gene flow can substantially influence key evolutionary processes and consequent persistence of recipient populations (Garant et al., 2007). Specifically, immigration can decrease inbreeding and increase local genetic variation, and may thereby increase population viability by alleviating expression of inbreeding depression and generating heterosis (leading to "genetic rescue", reviewed by Frankham, 1998; Tallmon et al., 2004) and/or by facilitating rapid adaptive evolution (leading to "evolutionary rescue", reviewed by Bell et al., 2019; Carlson et al., 2014; Gomulkiewicz & Shaw, 2013). Yet, at the same time, immigration could potentially disrupt the progress of local adaptation, and thereby decrease population viability by constraining local populations away from their potential fitness peak (i.e., migration load, Bolnick & Nosil, 2007; Garant et al., 2007; Lenormand, 2002). Understanding the diverse genetic impacts of immigration is therefore central to understanding evolutionary dynamics in metapopulation systems (Carlson et al., 2014; Garant et al., 2007; Lenormand, 2002; Reid et al., 2021), predicting fates of populations experiencing changing environments (Aitken & Whitlock, 2013), conservation of small inbred populations (Frankham, 2015), and optimal animal breeding (Fernández et al., 2012; Rudnick & Lacy, 2008).

Theoretical and empirical studies in all these research areas commonly make three standard assumptions regarding genetic properties of new immigrants into any focal population; that such immigrants are (1) outbred, (2) unrelated to all individuals in the existing recipient population at the time of arrival, and (3) unrelated to each other (Ballou, 1983; Hammerly et al., 2016; Ivy et al., 2009; Pemberton, 2008; Reid et al., 2006; Rudnick & Lacy, 2008; Slate et al., 2004; Wolak et al., 2018). These assumptions, which are often implicit rather than explicitly stated, underpin general predictions of the degree to which immigration will decrease inbreeding, cause heterosis, increase local genetic variation and impede local adaptation (Figure 1). This in turn implies that violations of any or all of the three assumptions could mean that immigration will not have the expected effects, and hence that evolutionary and population dynamic outcomes could differ from standard predictions. For example, inbred immigrants might directly experience inbreeding depression and/or be less beneficial for genetic rescue (Frankham, 2015; Ralls et al., 2020, but see Heber et al., 2013), while immigrants that are interrelated will probably cause less outbreeding and weaker heterosis than otherwise expected (Edmands, 2007; Frankham et al., 2011). Such immigrants will also import fewer novel genetic variants and thus have less impact on local adaptation and evolution. Furthermore, violations will cause errors in pedigree-based estimates of coefficients of inbreeding and kinship among immigrants, natives and their collective descendants. Resulting empirical estimates of key effects, including inbreeding depression, outbreeding depression, heterosis and additive genetic variance, might then be biased (Figure 1). However, despite their foundational role in theoretical and empirical evolutionary ecology, and potential impacts on conservation and breeding programmes (Fernández et al., 2012; Hammerly et al., 2016), the standard assumptions that new immigrants are outbred and unrelated to existing natives and to each other are rarely explicitly tested (Ivy et al., 2009; Robinson et al., 2019). This includes field studies that are central to empirical understanding of effects of inbreeding and microevolution in nature (Marr et al., 2002; Reid et al., 2006; Szulkin et al., 2007; Wolak et al., 2018; reviewed by Whiteley et al., 2015).

While the assumptions that immigrants are outbred and unrelated may be reasonable when immigrants originate from large panmictic populations, they might be regularly violated in natural metapopulations where small subpopulations are connected by dispersal. Here, immigrants into any focal subpopulation might originate from other small subpopulations, and consequently be as inbred, or even more inbred, than individuals in the focal recipient subpopulation (Chen et al., 2016). Immigrants could also be related to existing focal subpopulation members if there are repeated reciprocal dispersal events between locations, such that offspring or subsequent descendants of recent emigrants disperse back into their ancestors' original subpopulation. This pattern could be fueled if dispersal is

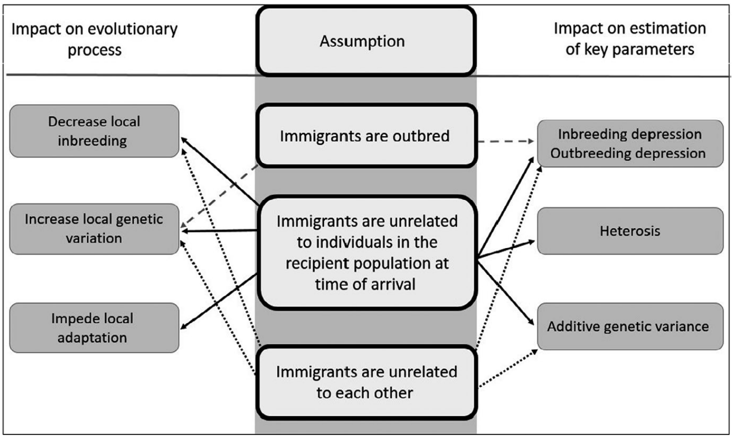


FIGURE 1 Potential main effects of violations of these assumptions on evolutionary effects of immigrants on the recipient population (left column) and on estimation of key parameters (right column). Dashed, solid and dotted lines of connecting arrows indicate the primary links involving the first, second and third assumptions, respectively [Colour figure can be viewed at wileyonlinelibrary.com]

heritable, increasing the probability that dispersers' offspring will also disperse (e.g., Doligez & Pärt, 2008). Immigrants might also be related to each other if individuals disperse alongside relatives and/or in other correlated ways, thereby potentially impacting genetic variation (Whitlock & McCauley, 1990). Indeed, sibling resemblance in aspects of dispersal has been observed in birds including great tits (*Parus major*, Matthysen et al., 2005), long-tailed tits (*Aegithalos caudatus*, Sharp et al., 2008), ortolan buntings (*Emberiza hortulana*, Dale, 2010), and house sparrows (*Passer domesticus*, Billing et al., 2012). Phenotype-dependent, and therefore genotype-dependent, habitat search and/or settlement could also result in clusters of related dispersers settling at the same location (e.g., in the warbler-finches *Certhidea olivacea* and *C. fusca*, Tonnis et al., 2005). Dispersal, and resulting immigration, might then be less random than is commonly assumed (Doligez & Pärt, 2008; Edelaar & Bolnick, 2012). Explicitly quantifying the degree to which new immigrants are in fact outbred and unrelated to natives and to each other, and adjusting subsequent analyses accordingly, could ultimately allow more precise and unbiased estimates of effects of inbreeding and outbreeding, and thereby help resolve ongoing debates regarding the various genetic effects of immigrants (Gomulkiewicz & Shaw, 2013; Reid et al., 2021). Such quantification could also contribute to general understanding of the degree of non-random immigration and the circumstances under which it occurs.

Testing the three standard assumptions (Figure 1) requires estimation of individual coefficient of inbreeding (*f*) and pairwise coefficients of kinship (*k*) in and among new immigrants and preexisting natives on a common quantitative scale. *K* between two individuals equals *f* of resulting offspring, meaning that *k* can be directly inferred from offspring *f* and vice versa (Falconer & Mackay, 1996, Supporting Information B). The coefficients *f* and *k* have traditionally been calculated from population pedigree data, and this remains a valuable approach (Galla et al., 2020; Nietlisbach et al., 2017; Pemberton, 2008). Expected values of *f* and *k* are calculated relative to a defined pedigree baseline "founder" population, typically taken as the set of individuals alive at the start of the study or breeding program (i.e., with unknown parents, Hogg et al., 2019; Lacy, 1989). Since subsequent immigrants are by definition not born within the focal population, their parents, grandparents and more distant ancestors are usually unknown. Standard pedigree analyses then include such immigrants in the defined base population, thereby making the standard assumptions that they are outbred and unrelated, thereby precluding direct estimation of *k* and *f* for the immigrants and potentially introducing influential pedigree errors if the standard assumptions are violated (Ivy et al., 2009; Rudnick & Lacy, 2008; Wolak & Reid, 2017).

Now, relatively high-density molecular genetic or genomic data can be used to estimate inbreeding and kinship (or relatedness) for any sampled individuals, including immigrants alongside existing natives (Wang, 2014). Such approaches can be used to validate founder relationships and correct and (re)construct pedigrees (Hammerly et al., 2016; Reid et al., 2014), thereby facilitating conservation and breeding programmes (reviewed in Blouin, 2003; e.g., Fernández et al., 2012; Ivy et al., 2009). However, challenges remain in computing, comparing and interpreting estimates of inbreeding and kinship for immigrants and natives on a common quantitative scale without introducing new assumptions. For example, many molecular genetic estimators utilize allele frequencies estimated from a reference population, which is often taken as a focal population sample (Wang, 2014). However, problems may arise because such focal reference allele frequencies may not represent immigrants, whose population of origin is often unknown and not sampled (Nietlisbach et al., 2018) and estimators can be sensitive to relatedness structure within the sample (Csilléry et al., 2006). Systems where both molecular genetic and pedigree data exist for focal population individuals can then be valuable, since measures of molecular marker homozygosity (and hence inbreeding and relatedness) among immigrants, existing natives and their potential offspring can be interpreted in the context of values of *f* and *k* for natives calculated relative to the defined pedigree baseline.

Long-term data from a song sparrow (*Melospiza melodia*) population resident on Mandarte Island, British Columbia, Canada, have proved valuable for examining the occurrence and consequences of inbreeding in the wild (Keller, 1998; Keller & Arcese 1998; Reid et al., 2014, 2015, 2016), and the quantitative genetic basis of life-history variation (Reid et al., 2011; Reid & Sardell, 2012; Wolak et al., 2018), including consequences of immigration (Marr et al., 2002; Reid & Arcese, 2020; Reid et al., 2021; Wolak et al., 2018). Recent analyses utilized complete multiyear pedigree data, where genetic parentage of focal Mandarte-hatched individuals was verified with very high statistical confidence (Nietlisbach et al., 2017; Reid et al., 2011, 2014, 2021; Sardell et al., 2010). However, as with all such studies, pedigree analyses invoked the three standard assumptions that new immigrants are outbred, unrelated to the native population at the time of arrival and unrelated to each other; but these assumptions have not been explicitly validated. Accordingly, we used genotypic data from 150–160 polymorphic microsatellite loci to estimate marker homozygosity and infer the degrees of inbreeding and kinship of immigrant song sparrows, for which ancestral pedigree data are unavailable, in relation to the multi-generational pedigree baseline for existing natives. We thereby evaluate to what degree the three standard assumptions hold, highlight how they could be tested in other systems, and consider the implications for estimates of key evolutionary processes and outcomes.

2 | MATERIALS AND METHODS

2.1 | Study system

Mandarte's song sparrow population has been studied intensively since 1975. In brief, all territories and nests were closely monitored, and all chicks reaching ≥6 days old were marked with unique combinations of coloured plastic and metal bands. All individuals that locally recruit as adults (age 1 year) are consequently individually identifiable (Arcese et al., 1992; Keller, 1998; Marr et al., 2002;

Smith et al., 2006; Wolak et al., 2018). The occasional immigrants to Mandarte are initially recognizable as unbanded adults present in spring, and are subsequently mist-netted and individually colour-banded. In total, 48 immigrants arrived during 1976–2016 (30 females, 18 males), with a mean of 1.2/year (range 0–4) with arrivals in 28 different years.

Since the total local population size is small (mean 77.2 ± 38.3 *SD* adults/year, range 12–159) and most immigrants successfully reproduced, the combined immigrants made a substantial genetic contribution to subsequent generations (Keller et al., 2001; Reid & Arcese, 2020; Reid et al., 2021; Wolak et al., 2018). Since Mandarte (latitude 48.6329°, longitude −123.2859°, 0.06 km^2) lies within ≤5 km of several other small islands (and 8 km away from Vancouver Island, 31,300 km^2), immigrants could potentially be inbred and/or related. The three standard assumptions (Figure 1) should consequently be explicitly verified.

2.2 | Genotypic data and measures of inbreeding and relatedness

All sparrows alive on Mandarte during 1993–2013 ($n = 3644$) were blood-sampled and initially genotyped at 13 highly polymorphic microsatellite markers to allow assignment of genetic parentage and compilation of a complete and accurate pedigree (all parents assigned with >99% individual-level confidence, Nietlisbach et al., 2017; Reid et al., 2014; Sardell et al., 2010; Wolak et al., 2018). This sample includes individuals that hatched on or immigrated to Mandarte during 1993–2013, alongside some surviving individuals that hatched or arrived in earlier years. Previous analyses of resulting pedigree data showed that mean *f* varied little across years (Reid et al., 2021), with no evidence of non-random mating with respect to kinship (i.e., inbreeding preference or avoidance, Keller & Arcese, 1998; Reid et al., 2015). The genetic marker data also verified the status of all presumed immigrants, since all adults alive in the breeding season before each immigrant's apparent arrival were excluded as their genetic parents with high confidence.

A sample of 2068 (56.8%) individuals was additionally genotyped at 150–160 autosomal microsatellite loci known to be polymorphic on Mandarte (mean genotyped loci per individual: 157.23 ± 2.80 *SD*; mean alleles per locus: 9.8 ± 5.2 *SD*, range 3–25; full details in Nietlisbach et al., 2015). This sample comprised most individuals alive during 1993–2009, and adult males alive during 2010–2013. This sampling was designed for other purposes, but for our current purposes we simply utilized all available marker data (full details in Supporting Information Figure A).

Several moment and maximum likelihood methods to estimate inbreeding and relatedness (or kinship) from genetic marker (e.g., microsatellite) data have been derived (Wang, 2014). However, such estimators are problematic to interpret when sampled individuals come from heterogeneous source populations, as with mixtures of immigrants and natives (Nietlisbach et al., 2018). This is partly because many such estimators use estimates of allele frequencies to attempt to distinguish homozygosity due to recent inbreeding and resulting identity by descent from identity by state (summarized in Keller et al., 2011; Slate et al., 2004; Wang, 2014). These allele frequencies can be estimated from available samples from single focal populations, but may differ for immigrants arriving from other unobserved populations (Fienieg & Galbusera, 2013; Wang, 2014). Performance and interpretation of such estimators can therefore depend on actual relatedness and metapopulation structure and resulting allelic frequency variation (e.g. Blouin, 2003; Oliehoek et al., 2006; Wang, 2011). Estimators can also be biased if there are numerous inbred or closely related individuals within the reference population (Wang, 2014), or if there is a high proportion of related individuals alongside unrelated individuals in the examined sample (Csilléry et al., 2006; Goudet et al., 2018). These conditions apply on Mandarte, where there is substantial inbreeding (Germain et al., 2018; Nietlisbach et al., 2017; Reid et al., 2014, 2015, 2016) and the origins of recent immigrants and associated subpopulation allele frequencies are unknown. Such estimators are consequently not appropriate for our current analyses.

Instead, basic estimates of inbreeding and kinship between individuals that do not explicitly incorporate allele frequencies can be obtained simply by computing marker homozygosity for observed immigrants and natives, and for their real or hypothetical offspring. Hence, to achieve our current objectives, we estimated each individual's degree of inbreeding as the proportion of genotyped microsatellite loci that were homozygous (i.e., number of homozygous loci divided by total genotyped loci, hereafter H_i). We estimated kinship between any two focal individuals as the proportion of loci that were observed to be homozygous in real offspring of observed pairings, or expected to be homozygous in potential offspring of hypothetical pairings (hereafter H_k; further explanations below). This relies on the point that *k* between two individuals equals *f* of their offspring (Supporting Information B). We did not standardize estimates by expected homozygosity at each locus (i.e., accounting for allele frequencies) because the expectation is unknown for immigrants, and previous analyses showed that unstandardized and standardized measures of homozygosity were highly correlated across non-immigrant individuals (correlation coefficient $r = 0.999$, Nietlisbach et al., 2017). A linkage map showed that the focal microsatellite loci are widely distributed across the genome (Nietlisbach et al., 2015), implying that observed marker homozygosity will broadly represent genome-wide homozygosity.

Alongside the advantages, there are also some challenges of using marker homozygosity to estimate inbreeding and kinship, which our analyses were designed to ameliorate. Resulting values of H_i and H_k presumably represent some degree of identity by state alongside identity by descent, and therefore do not quantitatively equal the pedigree-derived metrics *f* and *k* (Falconer & Mackay, 1996; Slate et al., 2004). Hence, to facilitate interpretation, we benchmarked values of H_i (and hence H_k) against pedigree *f*. We extracted values of *f* for genotyped "natives" (defined here as individuals whose parents and grandparents hatched on Mandarte and hence whose recent ancestors were not immigrants) calculated from the full Mandarte pedigree, and regressed H_i on f across these individuals. To estimate a value of H_i that broadly represents $f = 0$ relative to the defined pedigree baseline, we extracted the regression intercept with its 95% prediction interval. This intercept indicates what value of H_i implies that a song sparrow is "outbred" on the scale defined by the pedigreed Mandarte population. The prediction interval indicates the range of H_i values that could plausibly be observed in individuals with pedigree $f = 0$. We also extracted mean H_i for four further biologically meaningful values of f, corresponding to offspring of first-degree relatives (full-sibling or parent-offspring pairings, $f = 0.25$), second-degree relatives (e.g., half-siblings, $f = 0.125$), third-degree relatives (e.g., first cousins, $f = 0.065$), and fourth-degree relatives (e.g., an individual with its first cousin once removed, $f = 0.03125$). We did not directly extract H_i for individuals with pedigree $f = 0$ because the only genotyped individuals with $f = 0$ are immigrants and their offspring. These individuals are assigned $f = 0$ due to the three standard assumptions (i.e., that immigrants are outbred and unrelated), which is what we currently aim to test. Directly benchmarking H_i against f using these individuals would consequently be meaningless.

Using our methods, immigrants and their real and hypothetical offspring could potentially be less homozygous (i.e., lower H_i or H_k) than the estimated H_i value for $f = 0$ for the Mandarte pedigree baseline (i.e., the regression intercept). This could arise if immigrants originated from populations with different alleles, or different allele or genotype frequencies, than Mandarte, including less homozygosity at focal loci. This could in turn reflect higher local inbreeding and/or ascertainment bias (since the selected loci were all polymorphic on Mandarte, Nietlisbach et al., 2015). Our benchmarking therefore allows interpretation of H_i and H_k for immigrants on the scale of f and k defined relative to the baseline for the Mandarte population pedigree. H_i cannot be interpreted as a measure of the immigrants' f relative to their (unknown) population of origin, which cannot be less than 0. Our estimates of H_i that fall below the benchmark for $f = 0$ should also not be confused with negative values returned by estimators that treat inbreeding as a correlation coefficient rather than a probability of identity by descent (Wang, 2014).

Using genetic markers has the advantage that they capture variation in realized versus expected kinship arising from Mendelian inheritance (which is not captured by pedigree data, Keller et al., 2011). However, there is a well-known challenge that there will be non-trivial sampling variance around values of H_k (and hence relatedness between parents) estimated by observing H_i in single offspring, which is not fully resolved by using numerous loci. It has previously been emphasized that marker homozygosity is an imperfect measure of f at the individual level (Slate et al., 2004). We therefore focus on interpreting means across groups of individuals, which should be relatively precise and should not suffer from such severe sampling variance, and avoid over-interpreting single individual-level values or pair-level values inferred from single offspring.

2.3 | Testing assumption 1: Are immigrants outbred?

To test the assumption that immigrants were outbred relative to the native base population, we first calculated H_i for 18 immigrants that were alive on Mandarte at some point during 1993–2013 and hence were genotyped at 150–159 microsatellite loci (mean 155.10 ± 2.59 *SD*). These immigrants arrived in 11 different years during 1990–2013. We then calculated H_i for 1908 defined natives from the same period that were genotyped at 150–160 loci (mean 157.00 ± 2.82 *SD*; Supporting Information A). We used a Kolmogorov-Smirnov test to examine whether H_i for immigrants and natives probably derived from the same distribution defined by shape and location. Due to highly unbalanced sample sizes, we also directly tested whether the observed distribution of immigrant H_i differed from that which could be drawn by chance given the observed distribution of native H_i. Specifically, we randomly drew 18 observations of H_i from all natives, calculated the sample mean and variance across 1,000 iterations, and examined whether the observed mean and variance of the immigrants' H_i fell within the central 95% confidence interval of the simulated range. We additionally compared mean H_i estimated across the 18 immigrants to the benchmark for pedigree $f = 0$, and examined whether individual H_i values fell within the 95% prediction interval.

2.4 | Testing assumption 2: Are immigrants unrelated to natives?

We took two approaches to testing the assumption that immigrants are unrelated to natives at the time of arrival. Both use the conceptual point that mating between an unrelated immigrant and native (i.e., $k = 0$) would result in outbred (i.e., $f = 0$), and hence relatively heterozygous, offspring. First, we identified real offspring of observed immigrant-native pairings that had been genotyped at ≥150 microsatellite loci, and compared mean H_k across observed offspring of each immigrant (hereafter mean H_{k_o}) to the pedigree benchmarks. Offspring resulting from two known inbreeding events within immigrant lineages, where immigrant females bred with their own grandsons two years after arriving, were excluded from these analyses.

However, such analyses of real observed offspring obviously incompletely describe the kinship between new immigrants and all existing natives. Not all immigrants reproduced, or reproduced during the years in which offspring were genotyped at ≥150 loci. Of course, no immigrants reproduced with all opposite-sex natives, or with any same-sex natives. Immigrants might therefore have close relatives in the existing population that would not be detected through analyses of real offspring. Further, as noted above, since substantial Mendelian sampling variance in estimates of H_{k_o} should be expected, observation of a single real offspring with relatively high H_{k_o} does not necessarily mean that its immigrant and native parents were particularly closely related.

We circumvented these challenges through second analyses where we calculated the expected homozygosity of hypothetical offspring (hereafter H_{k_e}) that could be produced by all possible immigrant-native and native-native pairings among genotyped adults alive in the year in which each immigrant arrived (including same-sex pairs). H_{k_e} was calculated analytically, given the observed genotypes for each possible adult pair (Supporting Information C). We then calculated mean H_{k_e} across all hypothetical offspring of each focal immigrant and all coexisting natives, and compared these means to the pedigree benchmarks.

To illustrate the magnitude of sampling variance affecting H_k estimated across available microsatellite loci, we also simulated 20 hypothetical offspring for each possible pair by randomly drawing alleles from each parent, to obtain simulated offspring homozygosities (hereafter H_{k_s}, Supporting Information D). We extracted H_{k_e} and H_{k_s} values from the same successfully reproducing immigrant-native pairings for which H_{k_o} was also available, allowing direct comparison of mean H_{k_o}, mean H_{k_s} and H_{k_e}.

Finally, as an additional metric, we calculated the number of microsatellite alleles which each immigrant imported and which were not present in the existing Mandarte population at the time of its arrival.

2.5 | Testing assumption 3: Are immigrants unrelated to each other?

To test the assumption that immigrants are unrelated to each other, we calculated the expected homozygosity (H_{k_e}) of hypothetical offspring among all possible pairings of immigrants that had been genotyped at ≥150 microsatellite loci using the same methods as for the immigrant-native pairings (Supporting Information A, Figure A). This included all possible opposite-sex and same-sex pairings among immigrants, irrespective of their year of arrival. Since no real offspring resulting from immigrant-immigrant pairings were ever observed, such offspring could not be analysed directly. We then compared all pairwise values and mean H_{k_e} per focal immigrant to the pedigree benchmarks.

2.6 | Implementation

All analyses were implemented using R version 3.6.3 (R Core Team, 2018), using the tidyverse framework for data exploration, summary and visualization (Wickham et al., 2019) and package NADIV (Wolak, 2012) for pedigree analysis. All field data collection was approved by the University of British Columbia Animal Care Committee and conducted under banding permits from Environment and Climate Change Canada. Data are available from Dryad (Dickel et al., 2021).

3 | RESULTS

3.1 | Benchmarking, and homozygosity of immigrants versus natives

Based on the regression of H_i on pedigree *f* across the defined natives, predicted H_i benchmarks for $f = 0.25$, 0.125, 0.0625 and 0.03125, and hence for offspring of matings among first-, second-, third- and fourth-degree relatives, were 0.48, 0.40, 0.36 and 0.34, respectively. The intercept, representing $f = 0$, was 0.32 (Figure 2a). Prediction intervals spanned ranges of approximately ±0.081 around each prediction (Figure 2a). Individual H_i explained 35% of variation in pedigree *f* (adjusted $R^2 = 0.35$).

Mean H_i was 0.37 ± 0.05 *SD* (range 0.23–0.62) across the 1908 genotyped natives (and was quantitatively similar across 443 natives that survived to adulthood). Meanwhile, mean H_i was 0.33 ± 0.03 *SD* (range 0.27–0.39) across the 18 genotyped immigrants (Figure 2b). The distribution of H_i differed between the two groups (two sample Kolmogorov-Smirnov test, D = 0.48, $p < .01$). Additional simulations confirmed that both the mean and the variance of H_i were smaller in the 18 observed immigrants than in 18 randomly drawn natives (Figure 2c,d).

Mean H_i for the immigrants (0.33) was close to the estimated benchmark value of 0.32 for pedigree $f = 0$ (Figure 2a), and all individual H_i values were within the 95% prediction interval. Consequently, mean immigrant H_i is similar to that expected for locally outbred sparrows hatched on Mandarte, and individual H_i values lie within the predicted range of native H_i at $f = 0$.

3.2 | Relatedness of immigrants to natives

There was a total of 133 genotyped real offspring of immigrant-native pairings, produced by 12 of the 18 genotyped immigrants. Mean H_{k_o} across these offspring was 0.31 ± 0.04 *SD* (range 0.22–0.42, Figure 3a), corresponding closely to the benchmark of $H_i = 0.32$ for pedigree $f = 0$. Most immigrants' offspring were less homozygous than the genotyped natives, and than the immigrants themselves (Figure 2b). This implies that reproducing immigrants were typically unrelated to their native mates. However, one immigrant produced relatively homozygous offspring, broadly comparable to the estimated value for offspring of third-degree relatives (Figure 3a, individual 2008c). This implies that this immigrant was distantly related to its native mate. Observed variation in H_{k_o} (i.e., observed offspring homozygosity) was similar to the sampling variance evident in H_{k_s} (i.e., simulated offspring homozygosity), while H_{k_e} (i.e., expected offspring homozygosity) was quantitatively similar to mean H_{k_o} and mean H_{k_s}. This demonstrates substantial Mendelian sampling variance in H_{k_s} and hence H_{k_o}. Mean H_{k_o}, and H_{k_s}, and H_{k_e} are consequently the preferred values for inference.

In total, there were 26,626 possible native-native pairings and 1,850 possible immigrant-native pairings involving genotyped immigrants and natives alive in the immigrants' arrival years, representing 10 years between 1995 and 2013. On average, 88 ± 11% (range 65%–100%) of adults alive in each year were genotyped at 150–160 loci (Figure 4). It is therefore very unlikely that numerous natives to which an immigrant was related were excluded from analyses. Across all possible pairings, mean H_{k_e} was 0.38 ± 0.45 *SD* (range 0.27–0.65) for hypothetical offspring of native-native pairings and 0.30 ± 0.02 *SD* (range 0.24–0.40) for hypothetical offspring of immigrant-native pairings. Mean H_{k_e} for the hypothetical offspring of each immigrant was

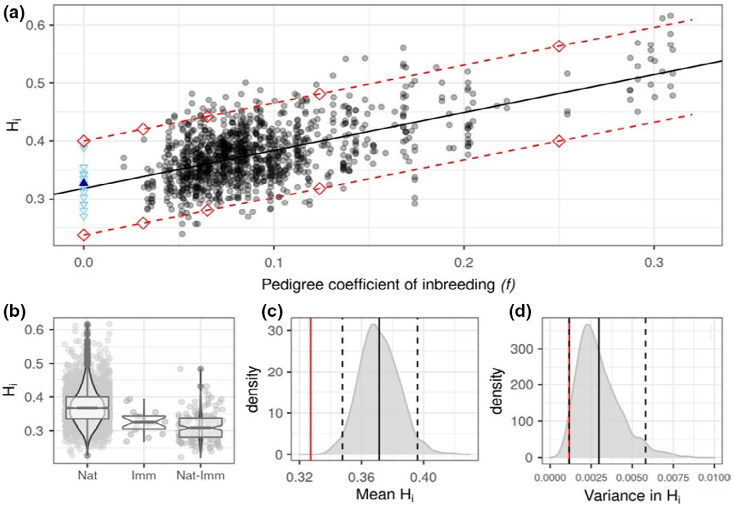


FIGURE 2 Summary of homozygosity of immigrants and natives. (a) Regression of individual homozygosity (H_i) on pedigree coefficient of inbreeding *(f)* across 1,180 defined natives (black line and points; adjusted $R^2 = 0.35$). Light blue hollow triangles indicate 18 immigrants, given the standard assumed inbreeding coefficient of $f = 0$. The dark blue filled triangle indicates the immigrants' mean (the median is quantitatively similar). Red diamonds indicate prediction intervals for biologically meaningful categories comprising offspring of first-, second-, third- and fourth-degree relatives and unrelated individuals ($f = 0$), connected by the red dotted line for visualization purposes. (b) Observed H_i of 1908 natives (Nat), 18 immigrants (Imm) and 133 immigrant-native offspring (Nat-Imm). Box plots indicate the median and quartiles. Violins indicate the full distribution. Points represent individuals, and are horizontally jittered to aid visibility. (c and d) Density distributions of mean (c) and variance (d) in H_i across 1000 random samples of 18 natives. Solid and dashed lines denote means and 95% confidence intervals, respectively. Red lines denote the observed mean and variance in H_i across the 18 observed immigrants [Colour figure can be viewed at wileyonlinelibrary.com]

typically considerably lower than mean H_{k_e} for the hypothetical offspring of natives alive in the same year (Figure 4). Mean H_{k_e} for most immigrants was also well below the benchmark for pedigree $f = 0$. This implies that the immigrants were typically unrelated to the natives alive at the time of arrival, relative to the local pedigree baseline. However, there is one clear exception, as one immigrant that arrived in 2008 would have produced relatively homozygous offspring (Figure 4; the same immigrant as noted in Figure 3a). Here, mean H_{k_e} fell between the benchmarks for third- or fourth-degree relatives' offspring, indicating that this immigrant was related to numerous existing natives (Figure 4). Further, mean H_{k_e} for one immigrant arriving in 2012 fell between the estimated benchmarks for $f = 0$ and fourth-degree relatives, implying that it might also have been distantly related.

Immigrants imported on average 36.2 ± 8.6 *SD* microsatellite alleles per individual that were not present in the genotyped sample of existing Mandarte adults alive at time of arrival (range 17–47, Supporting Information E). This directly indicates that immigrants were not closely related to the existing population and introduced novel genetic variation.

3.3 | Relatedness of immigrants to each other

Mean H_{k_e} of hypothetical offspring of all 153 possible immigrant-immigrant pairings was 0.30 ± 0.02 *SD* (range 0.25–0.41), and hence slightly below the benchmark for pedigree $f = 0$ (Figure 5). Yet, there was some variation, and two immigrants would have produced offspring of similar homozygosity to offspring of second-degree relatives on Mandarte (2012b and 2008b, Figure 5).

4 | DISCUSSION

The assumptions that immigrants are outbred, unrelated to the focal population at the time of arrival and unrelated to each other underpin considerable theoretical and empirical work in evolutionary ecology (Figure 1), and should therefore be explicitly validated. By combining unusually comprehensive pedigree and microsatellite marker data, we show that the three standard assumptions are broadly valid for our focal song sparrow system, which is a well-established model field system in evolutionary and conservation ecology (Arcese, 1989; Arcese et al., 1992; Keller, 1998; Reid et al., 2021; Smith et al., 2006; Wolak et al., 2018). Estimates of effects of immigration on key parameters such as the degree of inbreeding, heterosis and additive genetic variance, and resulting potential for genetic and evolutionary rescue, that utilize the standard assumptions will consequently be broadly valid. However, there are some minor deviations that illustrate the value of explicitly validating all three assumptions in the song sparrow system, and in other populations of interest.

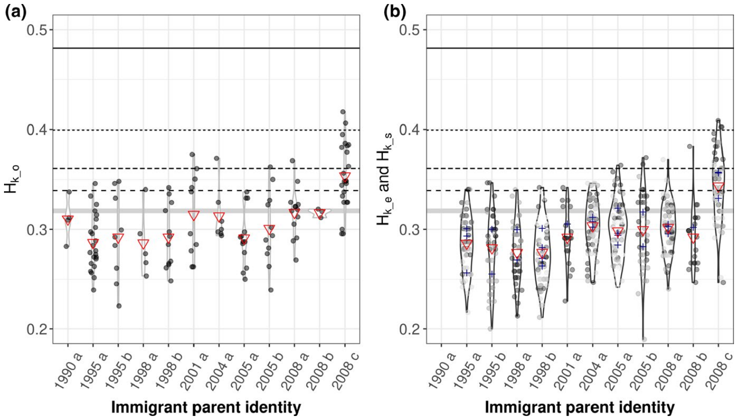
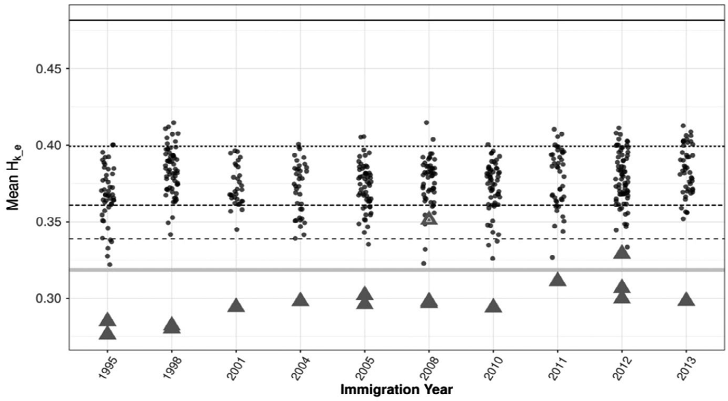


FIGURE 3 Summary of homozygosity of immigrants' offspring. (a) Observed homozygosity of observed offspring of each immigrant (H_{k_o}, grey points), ordered by the immigrant's arrival year with an individual identifier (a,b,c). Red triangles indicate mean H_{k_o} across each immigrant's observed offspring, and violins represent the full distributions. (b) Expected homozygosity (H_{k_e}, dark blue crosses) and simulated homozygosity (H_{k_s}, 20 realizations, grey points) of hypothetical offspring that could be produced by observed immigrant-native parents. Immigrant 1990a is excluded because no other individuals were genotyped at sufficient loci in 1990. Grey shades distinguish different pairings involving each immigrant. Red triangles indicate the mean across all simulated offspring of each immigrant. Grey points are horizontally jittered to aid visibility. Horizontal lines indicate predicted H_i benchmarks for values of pedigree f of 0.25 (solid), 0.125 (dotted), 0.0625 (dashed) and 0.01325 (spaced dashed). The grey band shows the benchmark value of H_i for pedigree $f = 0$ [Colour figure can be viewed at wileyonlinelibrary.com]



Immigrants	2/2	2/2	1/1	1/1	2/2	3/3	1/2	1/1	3/3	1/2
Natives	47/73	62/78	31/36	37/38	58/60	53/58	57/59	44/44	72/80	47/61

FIGURE 4 Mean expected homozygosity (H_{k_e}) across all possible hypothetical offspring that could be produced by each genotyped immigrant in its year of arrival (triangles) and by each native alive in the same year (points). The immigrant 2008c is indicated with an additional white triangle inside the grey triangle. Points are horizontally jittered to aid visibility. Horizontal lines indicate predicted H_i benchmarks for values of pedigree f of 0.25 (solid), 0.125 (dotted), 0.0625 (dashed) and 0.01325 (spaced dashed). The grey band shows the benchmark value of H_i for pedigree $f = 0$. Numbers below show the number of immigrants (*Imm*) that arrived in each year, and the number of adult natives (*Nat*) alive in each year, which were genotyped at ≥150 loci (left of slash) versus the total number (right of slash) in each category. Full underlying distributions of H_{k_e} are shown in Supporting Information Figure C

4.1 | Are immigrants outbred?

Pedigree data for immigrants and their ancestors, and knowledge of immigrants' origins, are rarely available unless field studies encompass entire meta-population systems (e.g., Billing et al., 2012; Niskanen et al., 2020) or immigration is facilitated by conservation programmes (e.g., Hasselgren et al., 2018). Consequently, pedigree coefficients of inbreeding (f) cannot typically be meaningfully

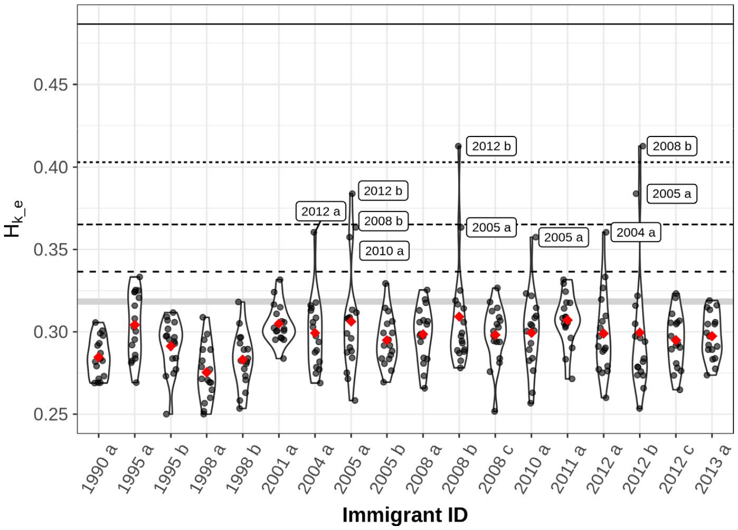


FIGURE 5 Expected homozygosity (H_{k_e}) of hypothetical offspring of immigrant-immigrant pairings. Grey points display H_{k_e} for each focal immigrant in hypothetical pairings with all other immigrants, and violins represent the full distributions. Points are horizontally jittered to aid visibility. Red diamonds indicate mean H_{k_e} for each immigrant. Horizontal lines indicate predicted H_i benchmarks for values of pedigree *f* of 0.25 (solid), 0.125 (dotted), 0.0625 (dashed) and 0.01325 (spaced dashed). The grey band shows the benchmark value of H_i for pedigree $f = 0$. Flags indicate the identity of the second immigrant parent of hypothetical offspring, indicating pairings with relatedness resulting in offspring comparable to fourth-degree relative offspring or closer [Colour figure can be viewed at wileyonlinelibrary.com]

directly calculated for immigrants, which are then either assumed to be outbred or excluded from analyses (e.g., Keller, 1998; Reid et al., 2014; Szulkin et al., 2007; Wolak et al., 2018). Our combined analyses of multilocus microsatellite homozygosity (H_i) and pedigree data showed that immigrants to Mandarte were on average less homozygous than existing natives, with mean H_i close to the estimated benchmark for pedigree $f = 0$, and individual values that fell within the 95% prediction interval. The genotyped immigrants can consequently be interpreted to be effectively outbred relative to the defined Mandarte population baseline.

Song sparrows are widespread and abundant across much of coastal British Columbia and more widely in North America, and there are multiple populations that are larger, less sedentary and/or less isolated than Mandarte's population within likely dispersal distance for passerine birds (e.g., house sparrow *Passer domesticus* metapopulation mean 22.9 ± 5.2 km, Tufto et al., 2005). Given Mandarte's relatively small size and low immigration rate, it is perhaps unsurprising that immigrants are on average less homozygous than existing population members.

This situation, and the corresponding assumption that immigrants are relatively outbred, are less likely to be valid when a focal population is not geographically isolated or particularly small compared to immigrants' source populations. Indeed, there are other small, inbred song sparrow populations on islands close to Mandarte, from which immigrants could potentially originate (Marr et al., 2002; Wilson & Arcese, 2008). The observed variation in H_i among immigrants to Mandarte could consequently reflect arrival of some relatively inbred individuals. However, the range of variation observed for immigrants does not exceed that observed for natives given any one value of pedigree *f*, or exceed simulated variation in offspring homozygosity. It could therefore simply reflect Mendelian and/or marker sampling variance. Future studies with much higher density mapped genomic data will allow tighter direct estimation of inbreeding coefficients of individual immigrants and natives, for example using runs of homozygosity (ROH). Such methods capture variance due to Mendelian inheritance and reduce the marker sampling variance, and thereby allow stronger individual-level rather than solely group-level inferences, and also give insights into histories of inbreeding events (Goudet et al., 2018; Hedrick & Garcia-Dorado, 2016; Kardos et al., 2015; Niskanen et al., 2020; Robinson et al., 2019).

4.2 | Are immigrants unrelated?

Mean expected H_{k_e} of hypothetical offspring of all possible immigrant-native pairings in each immigrant's year of arrival was typically substantially lower than mean H_{k_e} for hypothetical offspring of all possible native-native pairings. Consequently, the assumption that immigrants were effectively unrelated to the native population at the time of arrival was in most cases strongly validated. Immigrants would therefore produce effectively outbred offspring, introduce new genetic variation and probably cause heterosis, as commonly assumed (Charlesworth & Willis, 2009; Whiteley et al., 2015). Indeed, previous analyses of allelic diversity and heterozygosity at eight microsatellite markers showed that immigrants that arrived on Mandarte during 1989–1996 (i.e., largely pre-dating our current study) rapidly replenished neutral genetic variation lost through a severe population bottleneck in 1989 (Keller et al., 2001). This concurs with our current observation that recent immigrants introduced numerous new microsatellite alleles.

Further, our analyses show that immigrants' offspring would generally be even less homozygous than the benchmark for pedigree $f = 0$. Alongside the introduction of new alleles, this further implies that immigrants originated from populations with different allele frequencies than the observed Mandarte population. Their positive impact through reducing inbreeding and causing heterosis could consequently be even greater than inferred given the typical (often implicit) assumption that immigrants' offspring are outbred (i.e., $f = 0$) on a linear scale with existing natives (e.g., Wolak et al., 2018). In general, heterosis is often stronger with increasing genetic distance between mixed populations (as frequently demonstrated in agriculture, e.g., Springer & Stupar, 2007; Xiao et al., 1996; but see Jensen et al., 2018). Yet, risks of outbreeding depression in subsequent generations generally also increase with genetic and ecological differentiation (Frankham et al., 2011). Our evidence that immigrants are even less closely related to existing natives than typically assumed therefore implies that they could potentially have negative impact through outbreeding depression. Indeed, this is consistent with previous analyses that showed strong heterosis in F1 offspring of immigrant-native pairings in Mandarte's song sparrows, followed by outbreeding depression in the F2 generation that was apparent despite very small sample sizes (Marr et al., 2002).

Yet, despite the strong evidence that immigrants are typically unrelated to existing natives at arrival, one immigrant (arrived in 2008) was apparently somewhat related to the natives. A second immigrant (arrived in 2012) would also have produced offspring that were slightly more homozygous than the benchmark for pedigree $f = 0$. Such non-zero relatedness between immigrants and natives could potentially result from different dispersal patterns. First, it could reflect sequential reciprocal dispersal, where an emigrant's descendants disperse back to their ancestor's source population. Second, it could also arise if there is repeated directional immigration from the same source population across years. Non-zero relatedness between new arrivals and defined natives could then arise because the natives include descendants of previous immigrants. This would imply that immigrants that are apparently related to the native population are also related to at least one other immigrant. However, there was little evidence of such effects in our current data set. Specifically, there was little evidence that sampled immigrants were closely related to each other; rather the expected H_{k_e} of hypothetical immigrant-immigrant offspring was typically below the benchmark for pedigree $f = 0$. This implies that the immigrants come from a large source population and/or from different source populations, with no evidence of non-independent dispersal between related individuals, as observed in other passerine birds. For example, in house sparrows (*Passer domesticus*, Billing et al., 2012) and long-tailed tits (*Aegithalos caudatus*, Sharp et al., 2008) sibling pairs were detected among immigrants, in ortolan buntings (*Emberiza hortulana*) dispersal direction of siblings were similar (Dale, 2010), and in great tits (*Parus major*) siblings bred closer together than expected by chance (Matthysen et al., 2005).

4.3 | Implications and applications

The practical relevance (and fitness consequences) of mis-assigned relatedness between individuals has previously been demonstrated in the context of conservation breeding programs. For example in Attwater's prairie-chickens (*Tympanuchus cupido attwateri*), mean relatedness of parents was significantly reduced by using molecular relatedness information to identify optimal breeding pairs, leading to higher chick survival (Hammerly et al., 2016). In contrast, a simulation study based on data from captive parma wallabies (*Macropus parma*) found that molecular genetic estimates of relatedness would have little potential for improving genetic management by matching unrelated pairs for breeding, possibly because there were few unknown close relatives (Ivy et al., 2009). Indeed, general simulations have shown that, in the short term, offspring fitness would only be substantially increased when previously undetected close relatives (e.g., full siblings) are revealed (Rudnick & Lacy, 2008). Even here, impacts decrease over generations, meaning that long-term effects were minor. These insights from conservation genetics could be taken to imply that only recent inbreeding events are practically relevant to predicting population outcomes (Rudnick & Lacy, 2008, reviewed in Fienieg & Galbusera, 2013).

However, in natural populations experiencing regular immigration, knowledge of deviations from standard assumptions regarding immigrants might still substantially improve predictions of the effects of immigration on population demography and evolution. For example, such knowledge could reduce bias in estimates of inbreeding depression and heterosis, and thereby facilitate tests of population genetics theory and predictions of population viability (Frankham, 2015; Ralls et al., 2020). Since our song sparrow analyses revealed only relatively minor violations, the standard assumptions that immigrants are effectively outbred and unrelated to existing natives and to each other are reasonable starting points for analyses of evolutionary parameters and outcomes (as previously done, e.g. Marr et al., 2002; Wolak et al., 2018). Nevertheless, some subtleties can be incorporated into future pedigree-based analyses for our system, and also more widely. For example, offspring of apparently related immigrant(s) and/or their offspring could be excluded from analyses of heterosis, and non-linearities arising because other immigrants' offspring predominantly fell below the benchmark for pedigree $f = 0$ could be factored into analyses quantifying inbreeding depression.

Such adjustments will be most relevant in systems where immigrants are consistently related to pre-existing natives, and effects of deviations from the standard assumptions accumulate across generations. The ambition now should consequently be to evaluate the degree to which immigrants are typically relatively outbred and unrelated across other study systems and taxa. This would ultimately allow further broad evaluation of which assumptions are generally upheld or violated in relation to species life-history, geographical distributions, population structures and mating systems. This will in turn highlight circumstances where immigration, and hence underlying dispersal, is non-random with respect to relatedness (Doligez & Pärt, 2008; Edelaar & Bolnick, 2012). Our song sparrow analyses demonstrate how such advances can be achieved by combining pedigree and molecular genetic data. Current advances in acquiring and analysing genomic data in non-model organisms will soon mean that similar analyses can be achieved across diverse systems, allowing direct estimation of inbreeding and kinship without necessarily requiring long-term individual-based pedigree data.

ACKNOWLEDGEMENTS

We thank the Tsawout and Tseycum First Nations Bands for allowing access to Mandarte, everyone who contributed to long-term data collection, Natural Sciences and Engineering Research Council, Canada (NSERC), the Swiss National Science Foundation (recently P400PB-180870), the Research Council of Norway (SFF-III, project 223257) and the Norwegian University of Science and Technology (NTNU) for funding.

AUTHOR CONTRIBUTIONS

Lisa Dickel undertook the analyses and drafted the manuscript in collaboration with Jane M. Reid. Peter Arcese undertook and oversaw long-term field data collection. Pirmin Nietlisbach and Lukas F. Keller led genotyping and pedigree reconstruction. Pirmin Nietlisbach, Lukas F. Keller and Jane M. Reid contributed to fieldwork. All authors contributed substantially to conceptual development and manuscript editing.

DATA AVAILABILITY STATEMENT

The data used in this study are available from the Dryad digital repository at https://doi.org/10.5061/dryad.4j0zpc8c7 (Dickel et al., 2021).

ORCID

Lisa Dickel https://orcid.org/0000-0001-9412-5266
Pirmin Nietlisbach https://orcid.org/0000-0002-6224-2246
Henrik Jensen https://orcid.org/0000-0001-7804-1564

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. https://doi.org/10.1146/annurev-ecolsys-110512-135747

Arcese, P. (1989). Intrasexual competition, mating system and natal dispersal in song sparrows. *Animal Behaviour*, *38*(6), 958–979. https://doi.org/10.1016/S0003-3472(89)80137-X

Arcese, P., Smith, J. N. M., Hochachka, W. M., Rogers, C. M., & Ludwig, D. (1992). Stability, regulation, and the determination of abundance in an insular song sparrow population. *Ecology*, *73*(3), 805–822. https://doi.org/10.2307/1940159

Ballou, J. (1983). Calculating inbreeding coefficients from pedigrees. In C. M. Schoenwald-Cox, S. M. Chambers, B. MacBride, & L. Thomas (Eds.), *Genetics and conservation: A reference for managing wild animal and plant populations* (pp. 509–516). Benjamin Cummings.

Bell, D. A., Robinson, Z. L., Funk, W. C., Fitzpatrick, S. W., Allendorf, F. W., Tallmon, D. A., & Whiteley, A. R. (2019). The exciting potential and remaining uncertainties of genetic rescue. *Trends in Ecology and Evolution*, *34*(12), 1–11. https://doi.org/10.1016/j.tree.2019.06.006

Billing, A. M., Lee, A. M., Skjelseth, S., Borg, Å. A., Hale, M. C., Slate, J., Pärn, H., Ringsby, T. H., Saether, B.-E., & Jensen, H. (2012). Evidence of inbreeding depression but not inbreeding avoidance in a natural house sparrow population. *Molecular Ecology*, *21*(6), 1487–1499. https://doi.org/10.1111/j.1365-294X.2012.05490.x

Blouin, M. S. (2003). DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. *Trends in Ecology and Evolution*, *18*(10), 503–511. https://doi.org/10.1016/S0169-5347(03)00225-8

Bolnick, D. I., & Nosil, P. (2007). Natural selection in populations subject to a migration load. *Evolution*, *61*(9), 2229–2243. https://doi.org/10.1111/j.1558-5646.2007.00179.x

Carlson, S. M., Cunningham, C. J., & Westley, P. A. H. (2014). Evolutionary rescue in a changing world. *Trends in Ecology and Evolution*, *29*(9), 521–530. https://doi.org/10.1016/j.tree.2014.06.005

Charlesworth, D., & Willis, J. H. (2009). The genetics of inbreeding depression. *Nature Reviews Genetics*, *10*(11), 783–796. https://doi.org/10.1038/nrg2664

Chen, N., Cosgrove, E. J., Bowman, R., Fitzpatrick, J. W., & Clark, A. G. (2016). Genomic consequences of population decline in the endangered Florida scrub-jay. *Current Biology*, *26*(21), 2974–2979. https://doi.org/10.1016/j.cub.2016.08.062

Csilléry, K., Johnson, T., Beraldi, D., Clutton-Brock, T., Coltman, D., Hansson, B., Spong, G., & Pemberton, J. M. (2006). Performance of marker-based relatedness estimators in natural populations of outbred vertebrates. *Genetics*, *173*(4), 2091–2101. https://doi.org/10.1534/genetics.106.057331

Dale, S. (2010). Sibling resemblance in natal dispersal distance and direction in the Ortolan Bunting *Emberiza hortulana*. *Ibis*, *152*(2), 292–298. https://doi.org/10.1111/j.1474-919X.2010.01019.x

Dickel, L., Arcese, P., Nietlisbach, P., Keller, L., & Reid, J. M. (2021). Data for: Are immigrants outbred and unrelated? Testing standard assumptions in a wild metapopulation, Dryad, Dataset, https://doi.org/10.5061/dryad.4j0zpc8c7

Doligez, B., & Pärt, T. (2008). Estimating fitness consequences of dispersal: A road to "know-where"? Non-random dispersal and the underestimation of dispersers' fitness. *Journal of Animal Ecology*, *77*(6), 1199–1211. https://doi.org/10.1111/j.1365-2656.2008.01446.x

Edelaar, P., & Bolnick, I. D. (2012). Non-random gene flow: An underappreciated force in evolution and ecology. *Trends in Ecology and Evolution*, *27*(12), 659–665. https://doi.org/10.1016/j.tree.2012.07.009

Edmands, S. (2007). Between a rock and a hard place: Evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology*, *16*(3), 463–475. https://doi.org/10.1111/j.1365-294X.2006.03148.x

Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics*, 4th ed. Longman.

Fernández, J., Clemente, I., Amador, C., Membrillo, A., Azor, P., & Molina, A. (2012). Use of different sources of information for the recovery and genetic management of endangered populations: Example with the extreme case of Iberian pig Dorado strain. *Livestock Science*, *149*(3), 282–288. https://doi.org/10.1016/j.livsci.2012.07.019

Fienieg, E., & Galbusera, P. (2013). The use and integration of molecular DNA information in conservation breeding programmes: A review. *Journal of Zoo and Aquarium Research*, *1*(2), 44–51.

Frankham, R. (1998). Inbreeding and extinction: Island populations. *Conservation Biology*, *12*(3), 665–675. https://doi.org/10.1046/j.1523-1739.1998.96456.x

Frankham, R. (2015). Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. *Molecular Ecology*, *24*(11), 2610–2618. https://doi.org/10.1111/mec.13139.

Frankham, R., Ballou, J. D., Eldridge, M. D. B., Lacy, R. C., Ralls, K., Dudash, M. R., & Fenster, C. B. (2011). Predicting the probability of outbreeding depression. *Conservation Biology*, *25*(3), 465–475. https://doi.org/10.1111/j.1523-1739.2011.01662.x

Galla, S. J., Moraga, R., Brown, L., Cleland, S., Hoeppner, M. P., Maloney, R. F., Richardson, A., Slater, L., Santure, A. W., & Steeves, T. E. (2020). A comparison of pedigree, genetic and genomic estimates of relatedness for informing pairing decisions in two critically endangered birds: Implications for conservation breeding programmes worldwide. *Evolutionary Applications*, *13*(5), 991–1008. https://doi.org/10.1111/eva.12916

Garant, D., Forde, S. E., & Hendry, A. P. (2007). The multifarious effects of dispersal and gene flow on contemporary adaptation. *Functional Ecology*, *21*(3), 434–443. https://doi.org/10.1111/j.1365-2435.2006.01228.x

Germain, R. R., Arcese, P., & Reid, J. M. (2018). The consequences of polyandry for sibship structures, distributions of relationships and relatedness, and potential for inbreeding in a wild population. *American Naturalist*, *191*(5), 638–657. https://doi.org/10.1086/696855

Gomulkiewicz, R., & Shaw, R. G. (2013). Evolutionary rescue beyond the models. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1610), https://doi.org/10.1098/rstb.2012.0093

Goudet, J., Kay, T., & Weir, B. S. (2018). How to estimate kinship. *Molecular Ecology*, *27*(20), 4121–4135. https://doi.org/10.1111/mec.14833

Hammerly, S. C., de la Cerda, D. A., Bailey, H., & Johnson, J. A. (2016). A pedigree gone bad: increased offspring survival after using DNA-based relatedness to minimize inbreeding in a captive population. *Animal Conservation*, *19*(3), 296–303. https://doi.org/10.1111/acv.12247

Hasselgren, M., Angerbjörn, A., Eide, N. E., Erlandsson, R., Flagstad, Ø., Landa, A., & Norén, K. (2018). Genetic rescue in an inbred Arctic fox (*Vulpes lagopus*) population. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1875). https://doi.org/10.1098/rspb.2017.2814

Heber, S., Varsani, A., Kuhn, S., Girg, A., Kempenaers, B., & Briskie, J. (2013). The genetic rescue of two bottlenecked south island robin populations using translocations of inbred donors. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1752), https://doi.org/10.1098/rspb.2012.2228

Hedrick, P. W., & Garcia-Dorado, A. (2016). Understanding inbreeding depression, purging, and genetic rescue. *Trends in Ecology and Evolution*, *31*(12), 940–952. https://doi.org/10.1016/j.tree.2016.09.005

Hogg, C. J., Wright, B., Morris, K. M., Lee, A. V., Ivy, J. A., Grueber, C. E., & Belov, K. (2019). Founder relationships and conservation management: Empirical kinships reveal the effect on breeding programmes when founders are assumed to be unrelated. *Animal Conservation*, *22*(4), 348–361. https://doi.org/10.1111/acv.12463

Ivy, J. A., Miller, A., Lacy, R. C., & Dewoody, J. A. (2009). Methods and prospects for using molecular data in captive breeding programs: An empirical example using parma wallabies (*Macropus parma*). *Journal of Heredity*, *100*(4), 441–454. https://doi.org/10.1093/jhered/esp019

Jensen, C., Ørsted, M., & Kristensen, T. N. (2018). Effects of genetic distance on heterosis in a *Drosophila melanogaster* model system. *Genetica*, *146*(4–5), 345–359. https://doi.org/10.1007/s10709-018-0026-y

Kardos, M., Luikart, G., & Allendorf, F. W. (2015). Measuring individual inbreeding in the age of genomics: Marker-based measures are better than pedigrees. *Heredity*, *115*(1), 63–72. https://doi.org/10.1038/hdy.2015.17

Keller, L. F. (1998). Inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). *Evolution*, *52*(1), 240–250. https://doi.org/10.1111/j.1558-5646.1998.tb05157.x

Keller, L. F., Jeffery, K. J., Arcese, P., Beaumont, M. A., Hochachka, W. M., Smith, J. N. M., & Bruford, M. W. (2001). Immigration and the ephemerality of a natural population bottleneck: Evidence from molecular markers. *Proceedings of the Royal Society B: Biological Sciences*, *268*(1474), 1387–1394. https://doi.org/10.1098/rspb.2001.1607

Keller, M. C., Visscher, P. M., & Goddard, M. E. (2011). Quantification of inbreeding due to distant ancestors and its detection using dense single nucleotide polymorphism data. *Genetics*, *189*(1), 237–249. https://doi.org/10.1534/genetics.111.130922

Lacy, R. C. (1989). Analysis of founder representation in pedigrees: Founder equivalents and founder genome equivalents. *Zoo Biology*, *8*(2), 111–123. https://doi.org/10.1002/zoo.1430080203

Lenormand, T. (2002). Gene flow and the limits to natural selection. *Trends in Ecology and Evolution*, *17*(4), 183–189. https://doi.org/10.1016/S0169-5347(02)02497-7

Marr, A. B., Keller, L. F., & Arcese, P. (2002). Heterosis and outbreeding depression in descendants of natural immigrants to an inbred population of song sparrows (*Melospiza melodia*). *Evolution*, *56*(1), 131–142. https://doi.org/10.1111/j.0014-3820.2002.tb00855.x

Matthysen, E., Van De Casteele, T., & Adriaensen, F. (2005). Do sibling tits (*Parus major*, *P. caeruleus*) disperse over similar distances and in similar directions? *Oecologia*, *143*(2), 301–307. https://doi.org/10.1007/s00442-004-1760-7

Nietlisbach, P., Camenisch, G., Bucher, T., Slate, J., Keller, L. F., & Postma, E. (2015). A microsatellite-based linkage map for song sparrows (*Melospiza melodia*). *Molecular Ecology Resources*, *15*(6), 1486–1496. https://doi.org/10.1111/1755-0998.12414

Nietlisbach, P., Keller, L. F., Camenisch, G., Guillaume, F., Arcese, P., Reid, J. M., & Postma, E. (2017). Pedigree-based inbreeding coefficient explains more variation in fitness than heterozygosity at 160 microsatellites in a wild bird population. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1850), 20162763. https://doi.org/10.1098/rspb.2016.2763

Nietlisbach, P., Muff, S., Reid, J. M., Whitlock, M. C., & Keller, L. F. (2018). Nonequivalent lethal equivalents: Models and inbreeding metrics for unbiased estimation of inbreeding load. *Evolutionary Applications*, *12*(2), 266–279. https://doi.org/10.1111/eva.12713

Niskanen, A. K., Billing, A. M., Holand, H., Hagen, I. J., Araya-Ajoy, Y. G., Husby, A., Rønning, B., Myhre, A. M., Ranke, P. S., Kvalnes, T., Pärn, H., Ringsby, T. H., Lien, S., Sæther, B.-E., Muff, S., & Jensen, H. (2020). Consistent scaling of inbreeding depression in space and time in a house sparrow metapopulation. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(25), 14584–14592. https://doi.org/10.1073/pnas.1909599117

Oliehoek, P. A., Windig, J. J., Van Arendonk, J. A. M., & Bijma, P. (2006). Estimating relatedness between individuals in general populations with a focus on their use in conservation programs. *Genetics*, *173*(1), 483–496. https://doi.org/10.1534/genetics.105.049940

Pemberton, J. M. (2008). Wild pedigrees: The way forward. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1635), 613–621. https://doi.org/10.1098/rspb.2007.1531

R Core Team (2018) A language and environment for statistical computing. R Foundation for Statistical Computing, . https://www.R-project.org/

Ralls, K., Sunnucks, P., Lacy, R. C., & Frankham, R. (2020). Genetic rescue: A critique of the evidence supports maximizing genetic diversity rather than minimizing the introduction of putatively harmful genetic variation. *Biological Conservation*, *251*, Article 108784. https://doi.org/10.1016/j.biocon.2020.108784

Reid, J. M., & Arcese, P. (2020). Recent immigrants alter the quantitative genetic architecture of paternity in song sparrows. *Evolution Letters*, *4*(2), 124–136. https://doi.org/10.1002/evl3.162

Reid, J. M., Arcese, P., & Keller, L. F. (2006). Intrinsic parent-offspring correlation in inbreeding level in a song sparrow (*Melospiza melodia*) population open to immigration. *American Naturalist*, *168*, 1–13. https://doi.org/10.1086/504852

Reid, J. M., Arcese, P., Keller, L. F., Germain, R. R., Duthie, A. B., Losdat, S., Wolak, M. E., & Nietlisbach, P. (2015). Quantifying inbreeding avoidance through extra-pair reproduction. *Evolution*, *69*(1), 59–74. https://doi.org/10.1111/evo.12557

Reid, J. M., Arcese, P., Nietlisbach, P., Wolak, M. E., Muff, S., Dickel, L., & Keller, L. F. (2021). Immigration counter-acts local micro-evolution of a major fitness component: Migration-selection balance in free-living song sparrows. *Evolution Letters*, *5*(1), 48–60. https://doi.org/10.1002/evl3.214

Reid, J. M., Arcese, P., Sardell, R. J., & Keller, L. F. (2011). Additive genetic variance, heritability, and inbreeding depression in male extra-pair reproductive success. *American Naturalist*, *177*(2), 177–187. https://doi.org/10.1086/657977

Reid, J. M., Bocedi, G., Nietlisbach, P., Duthie, A. B., Wolak, M. E., Gow, E. A., & Arcese, P. (2016). Variation in parent-offspring kinship in socially monogamous systems with extra-pair reproduction and inbreeding. *Evolution*, *70*(7), 1512–1529. https://doi.org/10.1111/evo.12953

Reid, J. M., Keller, L. F., Marr, A. B., Nietlisbach, P., Sardell, R. J., & Arcese, P. (2014). Pedigree error due to extra-pair reproduction substantially biases estimates of inbreeding depression. *Evolution*, *68*(3), 802–815. https://doi.org/10.1111/evo.12305

Reid, J. M., & Sardell, R. J. (2012). Indirect selection on female extra-pair reproduction? Comparing the additive genetic value of maternal half-sib extra-pair and within-pair offspring. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1734), 1700–1708. https://doi.org/10.1098/rspb.2011.2230

Robinson, J. A., Räikkönen, J., Vucetich, L. M., Vucetich, J. A., Peterson, R. O., Lohmueller, K. E., & Wayne, R. K. (2019). Genomic signatures of extensive inbreeding in Isle Royale wolves, a population on the threshold of extinction. *Science Advances*, *5*(5), eaau0757. https://doi.org/10.1126/sciadv.aau0757

Rudnick, J. A., & Lacy, R. C. (2008). The impact of assumptions about founder relationships on the effectiveness of captive breeding strategies. *Conservation Genetics*, *9*(6), 1439–1450. https://doi.org/10.1007/s10592-007-9472-2

Sardell, R. J., Keller, L. F., Arcese, P., Bucher, T., & Reid, J. M. (2010). Comprehensive paternity assignment: Genotype, spatial location and social status in song sparrows. *Melospiza Melodia*. *Molecular Ecology*, *19*(19), 4352–4364. https://doi.org/10.1111/j.1365-294X.2010.04805.x

Sharp, S. P., Simeoni, M., & Hatchwell, B. J. (2008). Dispersal of sibling coalitions promotes helping among immigrants in a cooperatively breeding bird. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1647), 2125–2130. https://doi.org/10.1098/rspb.2008.0398

Slate, J., David, P., Dodds, K. G., Veenvliet, B. A., Glass, B. C., Broad, T. E., & McEwan, J. C. (2004). Understanding the relationship between inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. *Heredity*, *93*(3), 255–265. https://doi.org/10.1038/sj.hdy.6800485

Smith, J. N. M., Keller, L. F., Marr, A. B., & Arcese, P. (2006). *Conservation and biology of small populations: The song sparrows of Mandarte Island*. Oxford University Press.

Springer, N. M., & Stupar, R. M. (2007). Allelic variation and heterosis in maize: How do two halves make more than a whole? *Genome Research*, *17*(3), 264–275. https://doi.org/10.1101/gr.5347007

Szulkin, M., Garant, D., Mccleery, R. H., & Sheldon, B. C. (2007). Inbreeding depression along a life-history continuum in the great tit. *Journal of Evolutionary Biology*, *20*(4), 1531–1543. https://doi.org/10.1111/j.1420-9101.2007.01325.x

Tallmon, D. A., Luikart, G., & Waples, R. S. (2004). September). The alluring simplicity and complex reality of genetic rescue. *Trends in Ecology and Evolution*, *19*, 489–496. https://doi.org/10.1016/j.tree.2004.07.003

Tonnis, B., Grant, P. R., Grant, B. R., & Petren, K. (2005). Habitat selection and ecological speciation in Galápagos warbler finches (*Certhidea olivacea* and *Certhidea fusca*). *Proceedings of the Royal Society B: Biological Sciences*, *272*(1565), 819–826. https://doi.org/10.1098/rspb.2004.3030

Tufto, J., Ringsby, T. H., Dhondt, A. A., Adriaensen, F., & Matthysen, E. (2005). A parametric model for estimation of dispersal patterns applied to five passerine spatially structured populations. *American Naturalist*, *165*(1), https://doi.org/10.1086/426698

Wang, J. (2011). Coancestry: A program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Molecular Ecology Resources*, *11*(1), 141–145. https://doi.org/10.1111/j.1755-0998.2010.02885.x

Wang, J. (2014). Marker-based estimates of relatedness and inbreeding coefficients: An assessment of current methods. *Journal of Evolutionary Biology*, *27*(3), 518–530. https://doi.org/10.1111/jeb.12315

Whiteley, A. R., Fitzpatrick, S. W., Funk, W. C., & Tallmon, D. A. (2015). Genetic rescue to the rescue. *Trends in Ecology and Evolution*, *30*(1), 42–49. https://doi.org/10.1016/j.tree.2014.10.009

Whitlock, M. C., & McCauley, D. E. (1990). Some population genetic consequences of colony formation and extinction: genetic correlations within founding groups. *Evolution*, *44*(1940), 1717–1724.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., … Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Wilson, A. G., & Arcese, P. (2008). Influential factors for natal dispersal in an avian island metapopulation. *Journal of Avian Biology*, *39*(3), 341–347. https://doi.org/10.1111/j.0908-8857.2008.04239.x

Wolak, M. E. (2012). nadiv: an R package to create relatedness matrices for estimating non-additive genetic variances in animal models. *Methods in Ecology and Evolution*, *3*, 792–796. https://doi.org/10.1111/j.2041-210X.2012.00213.x

Wolak, M. E., Arcese, P., Keller, L. F., Nietlisbach, P., & Reid, J. M. (2018). Sex-specific additive genetic variances and correlations for fitness in a song sparrow (*Melospiza melodia*) population subject to natural immigration and inbreeding. *Evolution*, *72*(10), 2057–2075. https://doi.org/10.1111/evo.13575

Wolak, M. E., & Reid, J. M. (2017). Accounting for genetic differences among unknown parents in microevolutionary studies: how to include genetic groups in quantitative genetic animal models. *Journal of Animal Ecology*, *86*(1), 7–20. https://doi.org/10.1111/1365-2656.12597

Xiao, J., Li, J., Yuan, L., McCouch, S., & Tanksley, D. (1996). Genetic diversity and its relationship to hybrid performance and heterosis in rice as revealed by PCR-based markers. *Theoreticaland Applied Genetics*, *92*(6), 637–643. https://doi.org/10.1007/BF00226083

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Dickel, L., Arcese, P., Nietlisbach, P., Keller, L. F., Jensen, H., & Reid, J. M. (2021). Are immigrants outbred and unrelated? Testing standard assumptions in a wild metapopulation. *Molecular Ecology*, 30, 5674–5686. https://doi.org/10.1111/mec.16173

MOLECULAR ECOLOGY

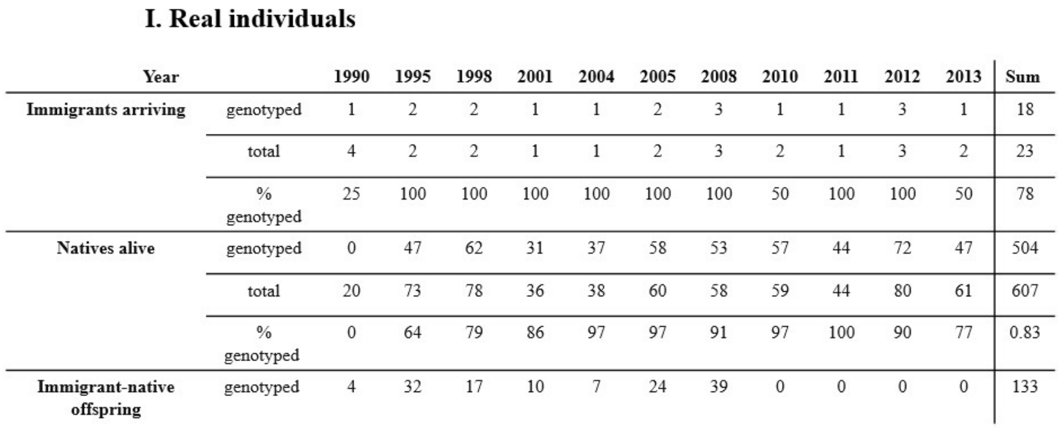
Supplemental Information for:

Are immigrants outbred and unrelated?
Testing fundamental assumptions in a wild meta-population

Lisa Dickel, Peter Arcese, Pirmin Nietlisbach, Lukas F. Keller,
Henrik Jensen, Jane M. Reid

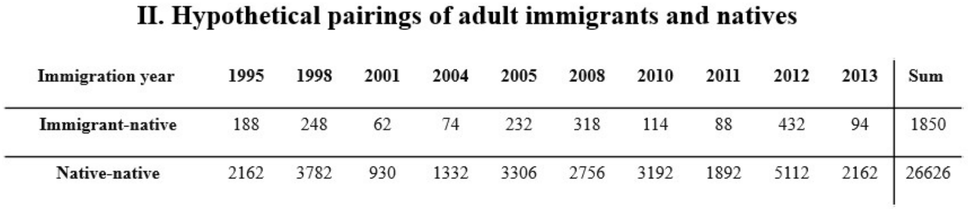
MOLECULAR ECOLOGY

A. Sample sizes



I. Real individuals

Year		1990	1995	1998	2001	2004	2005	2008	2010	2011	2012	2013	Sum
Immigrants arriving	genotyped	1	2	2	1	1	2	3	1	1	3	1	18
	total	4	2	2	1	1	2	3	2	1	3	2	23
	% genotyped	25	100	100	100	100	100	100	50	100	100	50	78
Natives alive	genotyped	0	47	62	31	37	58	53	57	44	72	47	504
	total	20	73	78	36	38	60	58	59	44	80	61	607
	% genotyped	0	64	79	86	97	97	91	97	100	90	77	0.83
Immigrant-native offspring	genotyped	4	32	17	10	7	24	39	0	0	0	0	133



II. Hypothetical pairings of adult immigrants and natives

Immigration year	1995	1998	2001	2004	2005	2008	2010	2011	2012	2013	Sum
Immigrant-native	188	248	62	74	232	318	114	88	432	94	1850
Native-native	2162	3782	930	1332	3306	2756	3192	1892	5112	2162	26626

Fig. A: Sample sizes for real and hypothetical individuals. Panel I. shows all real individuals included in the study during immigration years. Immigrants are shown relative to their year of arrival, while the number of natives indicates the number of natives alive in each year. The immigrant which arrived in 1990 was still alive when the genotyping started in 1993 and was therefore included in the sample. The total number of natives in the study was 1908, and some of these are not included in panel I because they were alive in years where no immigration occurred. Real offspring of each immigrant is listed in the year of the immigrants' arrival. The column of genotyped individuals refers to those being genotyped at 150-160 loci. The 18 genotyped immigrants comprised 9 females that arrived in years 1990, 1995, 1998, 2001, 2004, 2005 and 2008 and 9 males that arrived in years 1995, 2008, 2010, 2011, 2012 and 2013.

Panel II shows the resulting hypothetical parings among natives, between natives and immigrants and among immigrants. Because no other individuals were genotyped in 1990, the year does not appear in this table. In addition, there were 153 hypothetical pairings among immigrants.

MOLECULAR ECOLOGY

B. Relationship between kinship and inbreeding coefficient

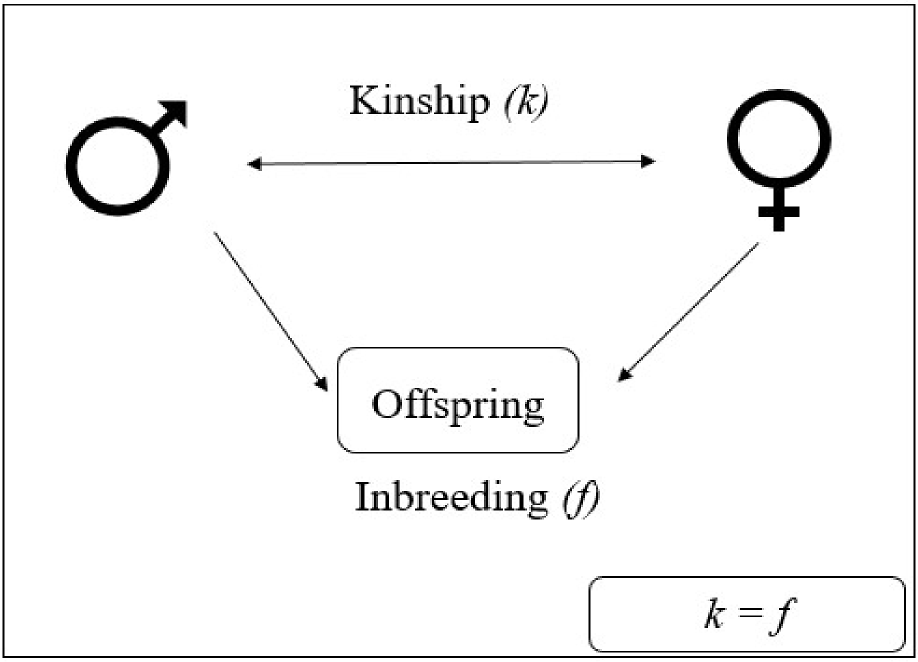


Fig. B: Illustration of the equivalence between the coefficient of kinship (*k*) between two individuals and the coefficient of inbreeding (*f*) of their combined (hypothetical) offspring. Because *k* = *f*, either one can be inferred if the other can be estimated (Falconer & Mackay 1996). This exact relationship is valid for pedigree based analyses. Inbreeding measures based on genetic data will be similar, but not exactly equal *f*, because they contain Mendelian and sampling variance which can cause deviation from the expected pedigree values.

MOLECULAR ECOLOGY

C. Analytically derived expected homozygosity

In order to assess relatedness between pairs of individuals we calculated expected homozygosity of hypothetical offspring using the R code below. To do so, we associated possible cases of alleles being the same (homozygote) or different (heterozygote) with their probability of homozygosity (i.e. 0, 0.25, 0.5 or 1) dependent on the parents' marker in each locus. We then calculated the mean to reach the overall expected homozygosity H_{i_e} and H_{k_e} of the hypothetical offspring across all loci.

C. 1 R code for calculating expected homozygosity of hypothetical offspring

```
library(dplyr)

subset_autosomal_loci <- subset_autosomal_loci  %>%

  mutate(exp_hz = case_when(id1_a == id1_b & id2_a == id2_b &

                                                          id1_a == id2_a & id1_b == id2_b ~ 1,

                          id1_a != id2_a & id1_a != id2_b & id1_b != id2_a & id1_b != id2_b ~ 0,

                          id1_a == id2_a & id1_b == id2_b & id1_a != id1_b |

                            id1_a == id1_b & id1_a == id2_a & id2_a != id2_b |

                            id1_a == id2_a & id1_b == id2_b & id1_a != id1_b |

                            id1_a == id2_b & id1_b == id2_a & id1_a != id1_b |

                            id1_a != id1_b & id1_b == id2_a & id2_a == id2_b |

                            id1_a != id1_b & id1_a == id2_a & id2_a == id2_b |

                            id1_a == id1_b & id1_b == id2_b & id2_a != id2_b ~ 0.5,

                          id1_a == id2_a & id1_a != id1_b & id1_a != id2_b |

                            id1_a == id2_b & id1_a != id1_b & id1_a != id2_a |

                            id1_b == id2_a & id1_a != id1_b & id1_b != id2_b |

                            id1_b == id2_b & id1_b != id1_a & id1_b != id2_a ~ 0.25))

subset_autosomal_test2ind %>%

                summarise(mean(exp_hz))
```

MOLECULAR ECOLOGY

Expected homozygosity of natives' and immigrants' offspring

Fig. C shows the full distributions of expected homozygosities of offspring among natives and between natives and immigrants in each year in which immigrants arrived in the focal population. This figure is an expanded version (with full distributions instead of solely mean values) of Fig. 4 in the main manuscript.

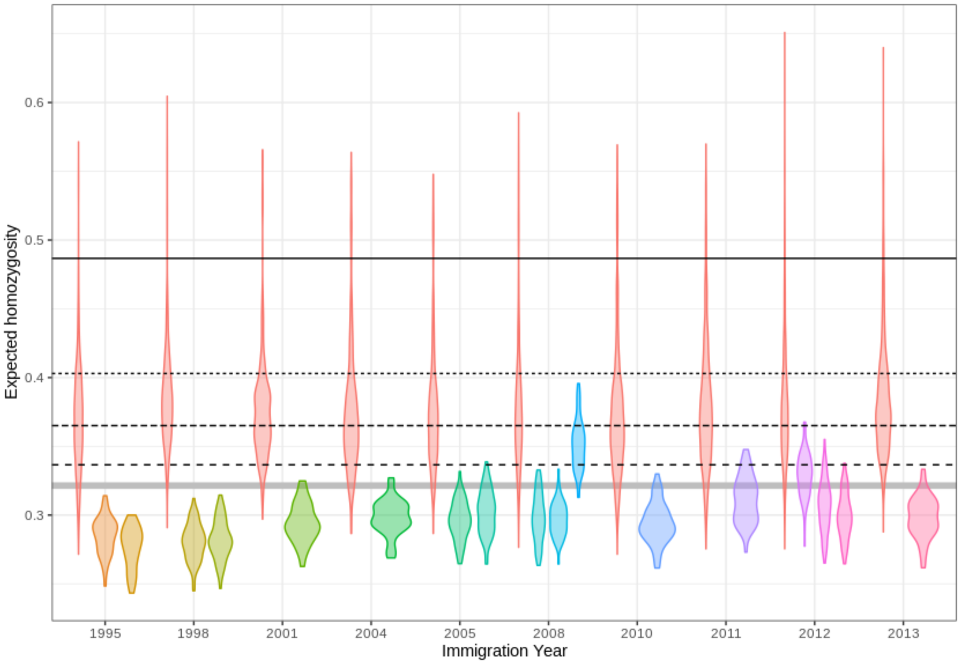


Fig. C: Full distribution of expected homozygosity of hypothetical offspring (H_{k_e}) within each year of immigrant arrival (while Fig. 4 in the manuscript displays only the mean for each individual). The red violins display the expected homozygosities resulting from native-native hypothetical pairings, while the differently colored violins display the distribution of expected homozygosity of immigrant-native pairings for each immigrant. Horizontal lines indicate predicted H_i benchmarks for values of pedigree f of 0.25 (solid), 0.125 (dotted), 0.0625 (dashed) and 0.01325 (spaced dashed). The grey band shows the benchmark value of H_i for pedigree f=0.

D. Simulated homozygosity

We also assessed homozygosity of hypothetical offspring using a simulation, in order to also assess sampling variance beside the expected mean which was already achieved in the analytical solution. We simulated by drawing randomly alleles from two individuals (the parents of the hypothetical offspring) twenty times. These simulations assume that alleles are inherited independently across loci. While this is not strictly true since some loci are on the same chromosome (Nietlisbach et al., 2015), linkage only affects the variance in relatedness but not its mean.

D 1. Simulation code

```
library(dplyr)
sim.fun <- function(pair_ids){
  subset_autosomal_2ind <- subset_autosomal_id %>%
      dplyr::filter(id %in% pair_ids)%>%
      dplyr::filter(id %in% hz_150_4$id)%>%
      select(-id)

    # keep only the columns without any NAs
    subset_autosomal_2ind <- subset_autosomal_2ind %>%
      select_if(~ !any(is.na(.)))

    # transpose the data frame: now long format
    subset_autosomal_2ind <- as.data.frame(t(subset_autosomal_2ind))

    # assign number of observations for generalization
    n <- nrow(subset_autosomal_2ind)
```

```
  subset_autosomal_2ind <-  subset_autosomal_2ind %>%
   rownames_to_column(var = 'Allelename')


 subset_autosomal_2ind$Allelename <- substr(subset_autosomal_2ind$Allelename, 1,
               nchar(subset_autosomal_2ind$Allelename)-1)


  subset_autosomal_test2ind$index <- rep(c('a', 'b'), n/2)


  subset_autosomal_ind <- pivot_wider(subset_autosomal_test2ind, id_cols = Allelename,
               values_from = c(V1, V2), names_from = index)


  # assigning random numbers
   subset_autosomal_test2ind$test1 <- rbinom(1, n = (n/2),  prob = 0.5)
  subset_autosomal_test2ind$test2 <- rbinom(1, n = (n/2),  prob = 0.5)


  # translate random 0/1 into the individual of which the allele is inherited
  subset_autosomal_2ind <- subset_autosomal_2ind %>%
   mutate(offsp_a = case_when(test1 == 1 ~ paste(V1_a),
                 test1 == 0 ~ paste(V1_b)),
       offsp_b = case_when(test2 == 1 ~ paste(V2_a),
                 test2 == 0 ~ paste(V2_b)))%>%
   mutate(offsp_hz = case_when(offsp_a == offsp_b ~ 1,
                  offsp_a != offsp_b ~ 0))
  mean(subset_autosomal_2ind$offsp_hz)
}
```

E. Imported alleles by immigrant

We calculated the number of alleles imported by each immigrant which were not present at the time of arrival in the present adult individuals and defined natives as one measure of relatedness, which are presented in the table below. If an immigrant was homozygous for a new allele, this was counted as inserting one new allele.

Table E1: Number of adults present in the population in each year that an immigrant arrived, and their total number of (unique) alleles summed across all genotyped loci; imported alleles per immigrant which were not present in the existing population at the time of the immigrants' arrival; and the proportion the imported alleles comprised in the year of arrival. As genotyping only started in 1993, no adult individuals were genotyped at the recorded time of arrival of the immigrant arriving in 1990. On average, immigrants imported 36.24±8.58 [41.1±9.3SD] microsatellite alleles which were not present in the genotyped sample of existing Mandarte adults at time of arrival and their range was 17-47 [22-55]. Numbers in square brackets refer to the same measures for only the adult natives considered at arrival of each immigrant (compared to otherwise all individuals present in the year of arrival).

Year	**Number of adults [natives] in year of arrival**	**Alleles in adult [native] population**	**Immigrant ID**	**Alleles imported by immigrant**	**Proportion** (imported/ present)
1995	76 [73]	1162 [1145]	1995 a	47 [48]	0.040 [0.042]
			1995 b	44 [44]	0.038 [0.038]
1998	83 [78]	1209 [1098]	1998 a	31 [42]	0.025 [0.038]
			1998 b	40 [53]	0.033 [0.048]
2001	42 [36]	1130 [1069]	2001 a	44 [47]	0.038 [0.044]
2004	47 [38]	1120 [988]	2004 a	42 [45]	0.037 [0.046]
2005	70 [60]	1161 [1024]	2005 a	35 [43]	0.030 [0.042]
			2005 b	25 [34]	0.021 [0.033]
2008	62 [58]	1001 [957]	2008 a	34 [40]	0.033 [0.042]
			2008 b	34 [42]	0.033 [0.044]
			2008 c	17 [22]	0.016 [0.024]

2010	66 [59]	1133 [973]	2010 a	34 [44]	0.030 [0.045]
2011	50 [44]	1002 [939]	2011 a	43 [46]	0.042 [0.049]
2012	91 [80]	1087 [1059]	2012 a	25 [25]	0.023 [0.024]
			2012 b	31 [32]	0.029 [0.030]
			2012 c	45 [48]	0.041 [0.045]
2013	73 [61]	1105 [1029]	2013 a	45 [52]	0.040 [0.051]

References

Nietlisbach, P., Camenisch, G., Bucher, T., Slate, J., Keller, L. F., & Postma, E. (2015). A microsatellite-based linkage map for song sparrows (Melospiza melodia). *Molecular Ecology Resources*, *15*(6), 1486–1496. doi: 10.1111/1755-0998.12414

Falconer, D.S & Mackay, T. F. C. (1996). Introduction to Quantitative Genetics (Fourth Edition) Harlow: Longman.

Paper II

Multi-generational fitness effects of natural immigration indicate strong heterosis and epistatic breakdown in a wild bird population

Lisa Dickel[1], Peter Arcese[2], Lukas F. Keller[3,4], Pirmin Nietlisbach[5], Debora Goedert[1], Henrik Jensen[1], Jane M. Reid[1,6]

Article type: Major Article

[1]Centre for Biodiversity Dynamics, Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway

[2]Department of Forest & Conservation Sciences, University of British Columbia, Vancouver, British Columbia, Canada

[3]Department of Evolutionary Biology & Environmental Studies, University of Zurich, Zurich, Switzerland

[4]Natural History Museum, University of Zurich, Zurich, Switzerland

[5]School of Biological Sciences, Illinois State University, Normal, Illinois, USA

[6] School of Biological Sciences, University of Aberdeen, Aberdeen, UK

Corresponding author: Lisa Dickel

email: dickel.lisa@gmail.com; lisa.dickel@ntnu.no

Keywords

lifetime reproductive success, outbreeding depression, epistasis, genetic dominance, gene flow, heterosis

Abstract

The fitness of immigrants and their descendants produced within recipient populations fundamentally underpins the genetic and population dynamic consequences of immigration. Immigrants can in principle induce contrasting genetic effects on fitness across generations, reflecting multi-faceted additive, dominance and epistatic effects. Yet, full multi-generational and sex-specific fitness effects of regular immigration have not been quantified within naturally structured systems, precluding inference on underlying genetic architectures and population outcomes. We used four decades of song sparrow *(Melospiza melodia)* life-history and pedigree data to quantify fitness of natural immigrants, natives and their F1, F2 and backcross descendants, and test for evidence of non-additive genetic effects. Values of key fitness components (including adult lifetime reproductive success and zygote survival) of F1 offspring of immigrant-native matings substantially exceeded their parent mean, indicating strong heterosis. Meanwhile, F2 offspring of F1-F1 matings had notably low values, indicating surprisingly strong epistatic breakdown. Further, magnitudes of effects varied among fitness components, and differed between females and males descendants. These results demonstrate that strong non-additive genetic effects on fitness can arise within weakly structured and fragmented populations experiencing frequent natural immigration. Such effects will substantially affect the net degree of effective gene flow and resulting local genetic introgression and adaptation.

Introduction

Many wild populations are highly fragmented, meaning that their dynamics and persistence substantially depend on the occurrence and consequences of dispersal and resulting gene flow that act to link sub-populations (Hastings and Harrison, 1994; Haddad et al., 2015; Saastamoinen et al., 2018; Millon et al., 2019). Predicting magnitudes of short-term genetic introgression, and resulting micro-evolutionary and population dynamic outcomes, then requires that immediate and multi-generational fitness consequences of natural immigration can be quantified and rationalized, but this is rarely achieved (Ingvarsson and Whitlock, 2000; Lenormand, 2002; Edelaar and Bolnick, 2012; Richardson et al., 2014; Charlesworth, 2018).

Long-standing theory and existing empirical studies highlight that the net fitness consequences of immigration can be complex and multi-faceted, reflecting combinations of additive and non-additive genetic effects acting across multiple generations, alongside environmental, ecological and behavioral effects (Ingvarsson and Whitlock, 2000; Whitlock, Ingvarsson, and Hatfield, 2000; Lenormand, 2002; Tallmon, Luikart and Waples 2004; Edmands, 2007; Edelaar and Bolnick, 2012; Grummer et al., 2022). If there is local adaptation, fitness of incoming immigrants could be lower than that of resident individuals in the recipient population (i.e., natives), mainly reflecting negative additive genetic effects (Fenster and Galloway 2000; Kawecki and Ebert, 2004; Richardson et al. 2014; Brady et al., 2019). Conversely, fitness of immigrants could exceed that of natives if immigrants originate from source populations with favorable developmental environments, or from larger populations experiencing less drift load and inbreeding depression (Lenormand, 2002; Guillaume and Perrin, 2006; Charlesworth and Willis, 2009; Brady et al., 2019).

Subsequently, F1 offspring of immigrant-native matings could have relatively high fitness due to increased heterozygosity and resulting expression of overdominance and directional dominance stemming from systematic masking of deleterious recessive alleles (Whitlock et al., 2000; Charlesworth and Willis, 2009). Introgression resulting from immigration can then effectively reverse the negative fitness effects of local inbreeding, even causing F1 fitness to exceed that of immigrant and/or native parents (broadly termed heterosis, Charlesworth and Willis, 2009). Yet, such positive fitness effects in F1 offspring can be reduced or eliminated if there are strong negative additive genetic effects of immigrant ancestry, or immediate incompatibilities between immigrant and native genomes, stemming from local adaptation, ecologically dependent isolation and/or non-random immigration (Lynch, 1991; Whitlock et al., 2000; Rhode and Cruzan 2005; Edelaar and Bolnick, 2012).

Substantially reduced fitness can also emerge in the subsequent F2 generation (i.e. offspring of F1-F1 matings), even given high fitness in parental F1s, representing delayed outbreeding depression (Lynch, 1991; Tallmon et al., 2004; Schneemann et al., 2020; Teixeira and Huber, 2021). This results from breakdown of co-adapted gene complexes through recombination, implying some form of epistasis (i.e. interactions among alleles at different loci). Combinations of dominance and epistatic effects will also shape fitness of backcrosses (e.g. offspring of F1-native matings) and subsequent descendants, which inherit re-distributed proportions of immigrant versus native genomes (Mather and Jinks, 1977; Lynch, 1991; Lynch and Walsh, 1998). Furthermore, all such effects could differ between females and males and among fitness components, reflecting genetic architectures and environmental sensitivities that differ between the sexes and/or between fitness components (e.g. Lawson Handley and Perrin, 2007; Delph and Demuth 2016; Wolak et al., 2018; Fraser et al., 2019; Svensson et al., 2019; Sardell and Kirkpatrick 2020; Ottenburghs 2022).

Given such multi-faceted potential effects, core ambitions spanning evolutionary, population and conservation biology should be to quantify sex-specific fitness components for immigrants and natives and their first- and second-generation descendants in wild populations, and infer underlying additive, dominance and epistatic genetic effects. Such work could reveal the broad genetic architectures of fitness and population differentiation, ultimately allowing prediction of magnitudes of effective gene flow and resulting genetic introgression (Ingvarsson and Whitlock 2000; Edmands, 2007; Hansen, 2013; Bell et al., 2019; Reid et al., 2021). Such advances require application of key aspects of population and quantitative genetics theory to multi-generational wild population datasets.

Here, line-cross theory provides a general conceptual framework to structure hypothesis tests and inferences (fig. 1, table 1, Supporting Information S1, Mather and Jinks 1977, Lynch, 1991; Lynch and Walsh 1998; Roff and Emerson 2006). Specifically, if genetic effects underlying fitness are purely additive, the mean fitnesses of F1 and F2 descendants by definition equal the mean across immigrants and natives. This is because F1s and F2s inherit half their (autosomal) genomes from immigrant and native ancestors (fig. 1A). Then, given additive and dominance effects with no epistasis, F1 fitness will deviate from the immigrant-native mean to some degree (for example generating positive heterosis). This deviation will then be halved in F2s, since half the increase in heterozygosity in F1s is lost (fig. 1B). Deviations from this expectation, for example when F2 fitness is lower than the mean of the F1 and the immigrant-native mean, then imply some form of epistasis. Epistasis can also be inferred from backcrosses, for example if the fitness of an F1-native backcross deviates from the F1-native mean (fig. 1C, Supporting Information S1). Overall, explicitly comparing fitness components between specific groups of immigrants, natives and their F1, F2 and backcross descendants can therefore inform on magnitudes and net directions of non-additive genetic effects (fig. 1, table 1), providing an empirically tractable window into the genetic basis of fitness and population differentiation.

Such line-cross principles have long been utilized in agricultural and experimental settings, where lines can readily be generated and crossed in broadly controlled or known environments (Mather and Jinks 1977; Fenster and Galloway 2000; Roff and Emerson 2006; Monson and Sadler 2010; Fu et al., 2014). Such studies commonly reveal evidence of local adaptation (e.g. Almeida et al., 2021), implying additive genetic divergence. They further reveal evidence of strong dominance effects manifested as positive heterosis in F1s (Fu et al., 2014), and/or of epistatic effects manifested as subsequent outbreeding depression (Monson and Sadler, 2010; Labroo, Studer, and Rutkoski, 2021). Positive heterosis in F1s has also been observed following introduction or movement of new individuals into highly inbred wild populations, generating ‘genetic rescue’ (Tallmon et al., 2004; Frankham, 2015; Åkesson et al., 2016; Weeks et al. 2017; Hasselgren et al., 2018; Bell et al., 2019). Here, F2 fitness is also widely suggested to typically be relatively high if source and recipient populations are ecologically similar and not strongly genetically diverged (Frankham et al., 2011; Ralls et al., 2018). Line-cross concepts can also apply to inter-specific crosses, explaining variable outcomes in F1s that can depend on environmental conditions (Rundle and Whitlock, 2001; Atsumi et al., 2021).

Yet, despite such extensive work in the contexts of highly inbred and/or highly diverged lines and populations, surprisingly few studies have explicitly quantified and compared the fitness of natural immigrants, natives and their descendants, and hence inferred underlying genetic effects on fitness, in structured or fragmented wild systems where sub-populations are regularly linked by natural dispersal (i.e. broadly defined meta-populations, Whitlock et al., 2013; D. Goedert, H. Jensen, L. Dickel, and J.M. Reid, unpublished manuscript). Comparisons between immigrants and natives have revealed diverse fitness differences (Armbruster, Bradshaw, and Holzapfel, 1997; Marr et al., 2002; Waser, Nichols, and Hadfield, 2013; Germain et al., 2017; Mobley et al., 2019; Barbraud and Delord, 2020; Martinig et al., 2020). However, fitness effects on subsequent generations are rarely explicitly quantified, even though multi-generational field datasets now exist. Since wild populations are increasingly fragmented, such studies are now required to fully understand and predict net impacts of natural connectivity and/or management orientated translocations (Tallmon et al., 2004; Frankham 2016; Bell et al., 2019). Because quantitative genetic effects are commonly highly environment-dependent (e.g. for inbreeding depression, heritability and heterosis; Crnokrak and Roff, 1999; Prill et al., 2014; Cheptou and Donohue, 2010; Fox and Reed, 2011), key effects must be quantified under fully natural rather than solely controlled environmental conditions (Whitlock et al., 2013).

However, the ambition to apply line-cross principles to dissect the fitness consequences of natural immigration also comes with clear challenges. Since key predictions explicitly concern the fitness of sequential generations (fig.1), fitness should be measured within rather than across generations. Specifically, this requires zygote-to-zygote (e.g. total number of fertilized eggs produced by each fertilized egg) rather than adult-to-adult (e.g. number of recruited offspring produced by each recruit) measures (Wolf and Wade, 2001; Orr, 2009). But, when field data are obtained from single focal populations, zygote-to-zygote fitness cannot be locally measured for immigrants in taxa with post-development dispersal. This is because immigrants cannot be present during pre-dispersal life-history stages. Direct comparisons involving immigrants are then restricted to adult (i.e. post-dispersal) fitness components, where differences due to genetic effects might be confounded by different developmental environments. Nevertheless, zygote-to-zygote fitness of natives, and of immigrants' descendants (F1s, F2s and backcrosses) can still be fully quantified in a broadly common natal environment. This in turn allows indirect inference of values of zygote-to-zygote fitness of immigrants that would be required to qualitatively change key biological conclusions. The biological plausibility of these inferred values can then be inspected (see Methods and Supporting Information 7). Comparisons involving backcrosses, F1s and natives can also be used to infer epistatic effects without directly requiring data on immigrant fitness (fig. 1, table 1, Supporting Information S1).

Further, if dispersal is heritable and/or shows inter-generational environmental effects, then dominance and epistatic effects on absolute fitness may be obscured because F1 and F2 descendants of immigrants may be more likely to emigrate, reducing their local fitness measured as contributions to the original immigrant's recipient population (e.g. Doligez and Pärt, 2008; Edelaar and Bolnick, 2012; Saastamoinen et al., 2018). However, since dispersal is typically predominantly restricted to specific life-history phases, fitness components that could be confounded by such effects can be isolated. Field studies that quantify and compare observable sequential components of fitness among immigrants, natives and their descendants can then provide valuable insights into overall forms and magnitudes of genetic effects that shape fitness and resulting introgression.

Accordingly, we applied line-cross principles to four decades of individual-based field data from a free-living song sparrow (*Melospiza melodia*) population receiving natural immigrants, and test for evidence of strong non-additive genetic effects on fitness components. This system has previously proved to be well suited for quantifying the relative fitness of natural immigrants, natives and their descendants (Marr, Keller and Arcese 2002; Reid et al., 2021). Specifically, we implemented sets of planned comparisons among fitness components of immigrants, natives and their F1, F2 and backcross descendants, thereby testing for dominance and epistatic effects manifested as heterosis and epistatic breakdown. We quantified the degree to which such effects differed between females and males and among fitness components, culminating in compound non-additive effects on overall fitness. We demonstrate notably strong components of F1 heterosis and F2 epistatic breakdown that were robust to biologically plausible values of unobservable pre-recruitment survival for immigrants. These results imply that strong multi-locus divergence exists in the focal system despite regular natural immigration, in turn shaping the progress of genetic introgression.

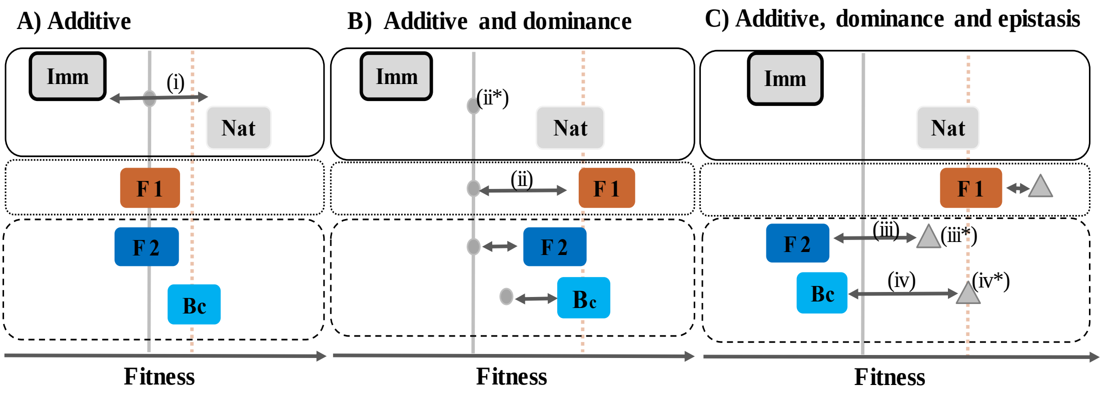


Figure 1: Conceptual illustration of expected fitness (x-axis) of distinct filial groups of individuals following immigration. (A) Expectation given solely additive genetic effects, where additive genetic values of immigrants in the recipient population environment are lower than natives, consistent with local adaptation. (B) Expectation given additive and dominance genetic effects, where F1 fitness deviates from the immigrant-native mean, here depicted as positive heterosis. (C) Expectation given additive, dominance and epistatic genetic effects, where F2 fitness deviates from the grand mean of the F1 and the immigrant-native mean, and backcross fitness deviates from the F1-native mean, depicted as epistatic breakdown. ‘Natives’ (Nat) are individuals with local ancestry; immigrants (Imm) have ancestors elsewhere; F1s are offspring of immigrant-native matings; F2s are offspring of F1-F1 matings; backcrosses (Bc) are offspring of matings between an F1 and one of the parental groups, here depicted as an F1-native mating. The y-axis dimension represents successive generations: solid boxes denote the parental generation (natives, immigrants), dotted boxes denote the first filial generation (F1s), and dashed boxes denote the second descendant generation (F2s, backcrosses). Shades match figures 2-7. On B and C, circles and triangles denote expectations given solely additive, or additive and dominance genetic effects, respectively. Arrows denote deviations from these expectations, given (B) dominance and (C) dominance and epistasis. Solid and dashed vertical lines indicate the immigrant-native and native-F1 means respectively. Underlying theory is summarized in Supporting Information S1. Numerals link to planned comparisons listed in table 1, where asterisks (*) denote means across groups. An equivalent figure for pre-dispersal fitness components where immigrants are unobservable is in Supporting Information S1.

Table 1: Summary of abbreviations for planned comparisons and means used to test for non-additive genetic effects on fitness components using line-cross theory. I, F1, F2, and Bc respectively denote immigrants and their F1, F2 and backcross descendants. NN denotes 'native-native' individuals, with eight locally-hatched great-grandparents (see Methods and Supporting Information S2). Figure element numerals link to fig.1 and fig. S1 (Supporting Information S1). Types indicate if each element refers to a planned comparison, a mean value calculated to be used in planned comparisons (highlighted by asterisks), or an alternative comparison when pre-dispersal immigrant data are unobservable (highlighted by daggers, Supporting Information S1).

Abbreviation	**Figure element**	**Type**	**Explanation and purpose**
ΔI-NN	(i)	Planned comparison	Difference in fitness component between defined immigrant and native groups
μ(I,NN)	(ii*)	Mean	Mean fitness component across the immigrant and native groups
ΔF1-μ(I,NN)	(ii)	Planned comparison	Difference in fitness component between the F1 group and the mean of the immigrant and native groups (i.e. the immigrant-native mean, μ(I, NN), used to test for heterosis
μ(F1,μ(I,NN))	(iii*)	Mean	Grand mean fitness component across the F1 group and the immigrant-native mean
ΔF1-NN		Alternative	Difference in fitness component

	(ii†)	comparison	between the F1 and native groups, used to test for heterosis without data for immigrants
$\mu(F1,NN)$	(iv*)	Mean	Mean fitness component across the F1 and native groups
$\Delta F2-\mu(F1,\mu(I,NN))$	(iii)	Planned comparison	Difference in fitness component between the F2 group and the grand mean of the F1 group and immigrant-native mean (i.e. $\mu(F1,\mu(I,NN))$), used to test for epistatic breakdown
$\Delta F2-\mu(F1,NN)$	(iii†)	Alternative comparison	Difference in fitness component between the F2 group and the mean of the F1 and native groups, used to test for epistatic breakdown without data for immigrants
$\Delta Bc-\mu(F1,NN)$	(iv)	Planned comparison	Difference in fitness component between the defined backcross group and the F1-native mean (i.e. $\mu(F1, NN)$), used to test for epistatic breakdown without requiring data for immigrants

Materials and Methods

Field data collection and pedigree construction

The small population of song sparrows inhabiting X̱OX̱ DEȽ (indigenous name, termed Mandarte island in English), BC, Canada (latitude 48.6329°, longitude 123.2859°, 0.06 km^2) lies within a natural meta-population. Here, numerous small and typically sedentary song sparrow populations inhabiting islands and mainland habitat patches are linked by occasional dispersal and resulting immigration (Smith et al., 1996, 2006; Marr, Keller and Arcese 2002; Reid, Arcese, and Keller, 2006). Analyses using 8 microsatellite markers showed that F_{ST} between various local populations exceeds zero, demonstrating fine-scale genetic structure at distances <10km (Wilson et al., 2011).

Song sparrow life histories have been quantified on Mandarte since 1975 (e.g. Arcese, Smith, and Hochachka, 1992; Smith et al., 2006). Both sexes can breed from approximately one year old (i.e. from the spring following the year of hatching), typically with social monogamy and biparental care (Gow et al., 2019). Pairs typically rear ≤3 broods of 1-4 chicks per summer, in open-cup nests built in demarcated territories. Each year from April, all occupied territories were mapped and closely monitored for breeding activity and outcomes. Nests were typically found during construction or incubation, and numbers of eggs laid and chicks hatched were recorded. All chicks surviving to approximately six days post-hatch were marked with unique combinations of metal and color bands. Hence, all locally hatched individuals are individually identifiable through field observations (Arcese et al., 1992; Keller, 1998; Marr et al., 2002; Smith et al., 2006; Wolak et al., 2018). The comprehensive annual territory monitoring meant that all individuals that locally survived to adulthood (i.e., ca. age 1 year) were identified, with local resighting probability of effectively one (Smith et al., 2006; Wilson et al., 2007). Mean local population size during 1976-2018 was 74.3±38.4SD adults/year (range 12-159). Adult sexes were attributed with high confidence based on reproductive behavior (e.g., male song, female incubation). Further, the comprehensive local banding means that individuals that first appear as unbanded adults in spring can be confidently defined as immigrants. A total of 48 immigrants arrived during 1976–2016 (30 females, 18 males, 1.2 individuals/year on average, Reid et al., 2021), and were mist-netted and color-banded to allow subsequent identification. All field data collection was approved by the University of British Columbia Animal Care Committee with banding permits from Environment and Climate Change Canada.

We used the comprehensive reproductive data to construct a full population pedigree by assigning all offspring produced since 1975 to their observed social parents (n= 6466). Further, all individuals banded since 1993 were blood-sampled and genotyped at up to 160 microsatellite markers to verify parentage and immigrant status (Nietlisbach et al., 2015). Analyses showed that all genetic maternities matched field-observed social mothers, while 28% of paternities were assigned to extra-pair males (Sardell et al., 2010). These paternity data were used to correct the pedigree as far as feasible, giving a complete, accurate genetic pedigree from 1993, social pedigree prior to 1989, and partially genetically verified pedigree through 1989-1992 (Sardell et al., 2010; Reid et al., 2014). The genetic analyses also confirmed that field-identified immigrants did not have genetic parents within the local population. They also showed that immigrants are typically unrelated to the population present at arrival (relative to the local pedigree baseline), and hence less related to the local residents than any residents are to each other (Dickel et al., 2021). To assign sexes for individuals that did not survive to adulthood, individuals banded since 1993 were genotyped at a sex-chromosome linked gene (*CHD1*, e.g., Postma et al., 2011).

In a preceding study, Marr et al. (2002) used 18 initial years of song sparrow data (1982-2000) to highlight intriguing indications of F1 heterosis and F2 breakdown (details in Supporting Information S3). However, their analyses assumed social rather than genetic parentage, and sample sizes were too small to draw statistically robust conclusions. Renewed analyses using the full available dataset and modern conceptual developments and statistical methods are now required to definitively quantify key multi-generational effects of immigration.

Group definitions

We assigned locally-hatched individuals to focal filial groups specified as natives, F1s, F2s or backcrosses using pedigree information on up to three generations of lineal ancestors (i.e., parents, grandparents and great-grandparents). We initially defined natives as locally-hatched individuals with four locally-hatched grandparents. However, to provide further definition of more distant ancestry, we divided the natives into three sub-groups defined by whether they had eight, seven or six locally-hatched great-grandparents. These groups respectively comprise focal individuals whose parent pairings were native-native, native-backcross or backcross-backcross (Supporting

Information S2). F1s and F2s were respectively defined as offspring of pairings between immigrants and natives (of any of the three sub-groups) and between two F1 individuals. Backcrosses were defined as offspring of pairings between F1s and natives, representing a native backcross (Supporting Information S2). The dataset also contained other types of individuals with eight known great-grandparents that did not fit the focal groups and were not the focus of specific current hypothesis tests (e.g. offspring of F1-F2 or F1-backcross pairings). All such individuals were pooled into an “other” group (Supporting Information S2). Since there were very few pairings between F1s and immigrants, these instances were included in the “other” group, rather than forming a separate immigrant backcross group. Meanwhile, individuals with at least one unknown great-grandparent due to missing data (other than because they had an immigrant parent or grandparent) were excluded. These individuals were predominantly hatched in early study years, and hence inevitably had insufficient known ancestry.

Fitness components

We extracted the set of sequential fitness components that together generate overall individual fitness. First, we quantified adult lifetime reproductive success (LRS) as the number of banded (i.e., 6 days old) offspring an adult (i.e., an individual that survived to age 1 year) produced during its lifetime. LRS was extracted for all adults hatched up to and including 2016. All individuals hatched in subsequent cohorts were excluded because some individuals were still alive, meaning that LRS is not yet fully known. We counted banded offspring rather than eggs laid as the best possible measure of an individual adult’s LRS despite slightly crossing generations (i.e., including early zygote and nestling survival, Supporting Information S3). This is because, since song sparrows readily replace lost clutches, individuals that lay most eggs are those whose breeding attempts repeatedly fail. The existence of an egg can therefore be conditional on early sibling mortality, meaning that total number of eggs is a somewhat misleading fitness component. We also quantified the two sub-components of LRS to examine underlying sources of variation: annual reproductive success (ARS) defined as the number of banded offspring that each adult produced during each year it was alive, and annual survival defined as whether or not each focal adult survived from one April in one year to April in the next year (Supporting Information S3). These sub-components were extracted for all adults alive up to and including 2018.

Second, we quantified local juvenile survival, defined as whether or not a banded (i.e., ~6 day old) chick locally survived to the next April census (i.e., approximately age 1 year, Supporting Information S3). This measure includes any unknown juvenile emigration, which cannot be separated from mortality. However, mean local juvenile survival has historically been quite high relative to adult survival, indicating relatively little emigration (Wilson and Arcese, 2008; Reid et al., 2021). To estimate sex-specific effects, we restricted data to cohorts hatched during 1993-2018, where all juveniles’ sexes have been genetically assigned.

Third, we quantified whether or not an egg (and hence a presumed zygote) survived from laying to banding (i.e. 6 day old chick, Supporting Information S3). To count numbers of eggs, clutch sizes were directly recorded as soon as possible after incubation commenced, with all eggs assumed to have been fertilized. When nests were not accessed until hatching, observed brood sizes of young chicks were assumed to match original clutch sizes. Nests in which eggs were never laid or which were found late were excluded from this analysis (3.9% of 2232 clutches during 1983-2019, widely distributed across groups; Supporting Information S4).

Adult fitness components (i.e., LRS, ARS and annual survival) were quantified for all focal groups of individuals including immigrants, and were therefore amenable to the full set of planned analyses. Local juvenile and zygote survival were quantified for all groups except immigrants which, by definition, do not exist on Mandarte through pre-recruitment (i.e. pre-immigration) life-history stages, thereby allowing restricted analyses.

Statistical analyses

We fitted a set of five separate generalized linear mixed models to test whether each of the five focal fitness components (as dependent variables) differed between the focal filial groups of individuals, and whether group effects differed between females and males. All models included fixed group effects, with eight levels for analyses of adult traits (i.e., immigrants, natives with three sub-groups, F1s, F2s, backcrosses and others) and seven levels for juvenile and zygote survival (with no immigrants). Models also included fixed sex effects (female versus male) and group by sex interactions, except for zygote survival since sexes are unknown for eggs (zygotes) that did not survive to genotyping or adulthood. However, analyses of hatchling sex ratios did not indicate any strong sex-biases in zygote survival probability (Supporting Information S4).

To adequately capture known patterns of variation with adult age, models for adult ARS and annual survival additionally included fixed effects of three age categories which we specified as young (age 1 year), middle (age 2-5 years) and old age (>5 years, following Keller, Reid, and Arcese, 2008; Wolak et al., 2018). Models also included random intercepts for natal year (zygote survival, juvenile survival, LRS) or current year (ARS, annual survival), and parent pair identity, to account for any non-independence of observations within cohorts, years and parent pairings (of focal individuals). Models for adult ARS and annual survival also contained random individual identity effects to account for repeated individual observations, and models for juvenile survival included random brood identity effects to account for non-independent survival of chicks banded in the same nest. Models for zygote survival were directly specified at the level of the clutch rather than individual eggs. The mixed group of ‘other’ individuals, which is not of direct interest, was retained in analyses to facilitate estimation of random year and cohort effects, thereby facilitating robust estimation of fixed effects for the main focal groups of interest. Models for adult LRS and ARS assumed Poisson error structures with log link functions and additive overdispersion, while models for adult, juvenile and zygote survival assumed binomial error distributions with logit link functions (Supporting Information S3).

Planned comparisons among groups

We fitted models in a Bayesian framework to facilitate post-hoc comparisons between fixed effects for specific groups and cross-group means while propagating uncertainty and reducing bias due to unbalanced sample sizes (fig. 1, table 1, Supporting Information S1). This approach allows us to explicitly test key predictions from line-cross theory, representing a major methodological advance beyond previous work (e.g. Marr et al., 2002, Supporting Information S3). Specifically, we calculated full posterior distributions for each of four planned comparisons (table 1), and examined whether these distributions spanned zero. First, to test whether measures of adult fitness differed between natives and immigrants (hereafter $\Delta_{\mathrm{I-NN}}$), we computed the posterior distribution of the difference in model-estimated effect sizes for the immigrant and native (specifically, native-native) groups. Second, to test for differences between F1s and the mean of their parent groups, and hence test for heterosis, we computed the posterior distribution of the mean of the immigrant and native-native group effect (hereafter $\mu_{(\mathrm{I,NN})}$), then computed the posterior distribution of the difference from the F1 effect (hereafter $\Delta_{\mathrm{F1}-\mu(\mathrm{I,NN})}$). Third, to test for epistatic breakdown in F2s, we computed the posterior distribution of the mean of the $\mu_{(\mathrm{I,NN})}$ and F1 effects (hereafter $\mu_{(\mathrm{F1},\mu(\mathrm{I,NN}))}$), then computed the posterior distribution of the difference from the F2 effect (hereafter $\Delta_{\mathrm{F2}-\mu(\mathrm{F1},\mu(\mathrm{I,NN}))}$). Fourth, to further test for epistatic breakdown without requiring data for immigrants, we computed the posterior distribution of the mean of the native (NN) and F1 effects (hereafter $\mu_{(\mathrm{F1,NN})}$) and then computed the difference from the backcross effect (hereafter $\Delta_{\mathrm{Bc}-\mu(\mathrm{F1,NN})}$). To explicitly test for sex-specific effects, we computed all four comparisons (i.e. $\Delta_{\text{I-NN}}$, $\Delta_{\text{F1-}\mu(\text{I,NN})}$, $\Delta_{\text{F2-}\mu(\text{F1},\mu(\text{I,NN}))}$, $\Delta_{\text{Bc-}\mu(\text{F1,NN})}$, table 1) separately for males and females. To test for sex-differences, we computed the posterior distributions of the differences between the female and male effects, where positive differences imply greater positive effects in females.

The comparisons $\Delta_{\text{I-NN}}$, $\Delta_{\text{F1-}\mu(\text{I,NN})}$ and $\Delta_{\text{F1-}\mu(\text{F1-}\mu(\text{I,NN}))}$ cannot be computed for juvenile and zygote survival, because, by definition, there are no local data for immigrants. We therefore implemented a further set of comparisons where differences were computed relative to natives rather than immigrant-native means (table 1). Specifically, to test for heterosis, we computed the posterior distribution of the difference between the F1 and native-native effects (hereafter $\Delta_{\text{F1-NN}}$). To test for epistatic breakdown, we computed the posterior distribution of the mean of the native-native and F1 effects (hereafter $\mu_{(\text{F1,NN})}$), then computed the difference from the F2 effect (hereafter $\Delta_{\text{F2-}\mu(\text{F1,NN})}$), table 1, Supporting Information S1).

Additional analyses

To further substantiate the evidence for heterosis and epistatic breakdown given incomplete data for immigrants and the potential for environmentally confounded values for adult fitness components, and to examine possible mechanisms, we undertook two further sets of analyses. First, we examined whether hypothetical values for immigrants that would be required for observed effects in F1s and F2s to arise given pure additive and/or dominance, rather than additional dominance and epistasis respectively, are biologically plausible or consistent with previously estimated additive genetic effects (Wolak et al., 2018; Reid et al., 2021; detailed explanations in Supporting Information S7).

Second, we examined the degree to which apparent heterosis could be explained simply as reduced inbreeding depression. Considerable among-individual variation in pedigree coefficient of inbreeding (*f*) exists in the focal population, with strong inbreeding depression (particularly in juvenile survival and adult reproductive success, Keller, 1998; Reid et al., 2014; Wolak et al., 2018). Our current main analyses deliberately did not include regressions of fitness components on *f*, or hence explicitly estimate inbreeding depression. Such regressions would directly control for part of the main group effects that we currently aim to estimate, since mean *f* differs among groups. However, to evaluate such effects, we re-fitted models including additional sex-specific regressions on *f* (Supporting Information S8).

Scale transformations and compound effects

All planned comparisons were initially computed on modeled latent scales (i.e. log or logit scale), which is arguably the scale on which additive and non-additive genetic effects underlying non-Gaussian traits are most appropriately quantified (de Villemereuil et al., 2016). Indeed, it is well known that additive effects on latent scales can become non-additive on phenotypic scales, potentially generating phenotypic dominance and epistasis (Mather and Jinks 1977). However, to facilitate biological interpretation, we back-transformed all posterior distributions of model estimates and planned comparisons onto phenotypic scales.

Then, to draw the best possible inference on magnitudes of sex-specific heterosis and epistatic breakdown in overall fitness, we multiplied through the back-transformed estimates of key sequential fitness components. Specifically, we multiplied posterior distributions for zygote and juvenile survival probabilities and adult lifetime reproductive success pertaining to focal individuals from each focal group, thereby generating the posterior distribution of the expected number of next-generation 6 day-old chicks produced by each zygote (i.e. egg; Supporting Information S3). This post-hoc analysis measures fitness as close to zygote-to-zygote as feasible, utilizing all available data for different fitness components (which spanned different years and cohorts and hence individuals). Such post-hoc posterior multiplications are valid because zygote survival, juvenile survival and adult lifetime reproductive success are effectively independent non-overlapping traits, with no shared parameters across the underlying models.

To further substantiate inferences on non-additive effects on overall fitness given the inevitable absence of early-life data for immigrants, we computed the posterior distributions of the comparisons $\Delta_{\mathrm{F1\text{-}NN}}$, $\Delta_{\mathrm{F2\text{-}}\mu(\mathrm{F1,NN})}$, and $\Delta_{\mathrm{Bc\text{-}}\mu(\mathrm{F1,NN})}$ separately for females and males, and computed the posterior distributions for the between-sex differences as described above. To exclude possible confounding effects of any group-specific juvenile emigration, we repeated these post-hoc analyses using constant sex-specific rather than group-specific juvenile survival (Supporting Information S6). Finally, to encompass immigrants, we repeated analyses using previously estimated additive genetic effects of immigrants on juvenile survival (Supporting Information S6).

Implementation

All analyses were implemented in R version 4.1.2 (Team, 2020) using the tidyverse framework for data handling (Wickham et al., 2019), package MCMCglmm for Bayesian analyses (Hadfield, 2010), and package tidybayes for visualization of estimates (Kay, 2021).

All models used substantially uninformative priors on fixed effects (normal distribution with mean 0 and variance 1000) with parameter expanded priors for variance components. Residual variances were estimated for adult LRS and ARS and zygote survival (the latter modelled as a clutch-level multinomial), and fixed to one by convention for adult and juvenile survival (since residual variance is not directly identifiable, Hadfield, 2010). Approximately 2000 effective posterior samples for focal parameters were retained, with low auto-correlation and no convergence issues. Posterior distributions were summarized with posterior means and 95% credible intervals (CIs), along with the proportions of values that exceeded zero for planned comparisons. To facilitate biological interpretation results are primarily presented on back-transformed phenotypic scales. Posterior summaries and group sample sizes are provided on Figures 2-7. Latent and phenotypic scale estimates are provided in Supporting Information S4, demonstrating that back-transformation did not alter key conclusions. To facilitate easy reference, all key abbreviations and definitions are summarized in Table 1.

Results

Adult fitness components

In females, LRS did not differ markedly between immigrants and natives (fig. 2A), with a posterior distribution of the difference ($\Delta_{\mathrm{I-NN}}$) that substantially overlapped zero (fig. 2C). Meanwhile, in males, immigrants tended to have lower LRS than natives (fig. 2B); approximately 90% of the posterior distribution for $\Delta_{\mathrm{I-NN}}$ was negative (fig. 2D). However, there was only moderate evidence that $\Delta_{\mathrm{I-NN}}$ was more negative in males than females (fig. 2E).

In both sexes, adult F1 offspring of immigrant-native matings tended to have higher LRS than either immigrants or natives (fig. 2A,B), and hence than the immigrant-native mean (fig. 2C,D). These effects were weak in females, where the posterior distribution for $\Delta_{\mathrm{F1}-\mu(\mathrm{I,NN})}$ substantially overlapped zero (fig. 2C), and furthermore the backcross-backcross group of natives had relatively high LRS (fig. 2A). However, F1 males had notably high LRS; the posterior mean for the F1 effect substantially exceeded those for all native and immigrant groups (fig. 2B), generating a posterior distribution for $\Delta_{\mathrm{F1}-\mu(\mathrm{I,NN})}$ that entirely exceeded zero (fig. 2D). On average, adult F1 males produced 6.6 (95%CI 2.0; 12.9) more offspring over their lifetimes than the immigrant-native mean, representing a large biological effect compared to the grand mean male LRS of 8.5±9.9SD (Supporting Information S5). Consequently, there was fairly strong evidence that $\Delta_{\mathrm{F1}-\mu(\mathrm{I,NN})}$ was greater in males than females; 92% of the posterior distribution of the sex-specific difference was negative (fig. 2E).

The adult F2 grand-offspring of immigrant-native matings through both parental lines (i.e. offspring of F1-F1 matings) had low LRS compared to most other groups in both sexes (fig. 2A), and hence compared to the grand mean across F1s and the immigrant-native mean (i.e. $\mu_{(F1,\mu(I,NN))}$, fig. 2B). Specifically, 78% and 99% of the posterior distributions for $\Delta_{F2-\mu(F1,\mu(I,NN))}$ were negative in females and males respectively (fig. 2C,D), with no strong evidence of a sex difference (fig. 2E). On average, adult F2 females and males respectively produced -2.2 (95%CI -7.6; 5.9) and -4.0 (95%CI -7.6; -0.7) fewer offspring over their lifetimes than the sex-specific values of $\mu_{(F1,\mu(I,NN))}$, again representing substantial biological effects. These detrimental F2 effects are evident despite the small total numbers of F2s that survived to be included in analyses of adult LRS (fig. 2A,B).

Finally, in both sexes, adult backcross offspring of F1-native matings had similar LRS to the natives (fig. 2A). Yet, due to the high LRS of F1s, backcrosses of both sexes had lower LRS than $\mu_{(F1,NN)}$, resulting in negative values of $\Delta_{Bc-\mu(F1,NN)}$ (fig. 2C,D). This difference was somewhat stronger in males than females; 96% and 77% of the posterior distributions for $\Delta_{Bc-\mu(F1,NN)}$ were negative respectively, but the posterior distribution of the difference between the sexes substantially overlapped zero (fig. 2E).

Adult annual traits

The basis of observed differences in LRS between focal groups is revealed by considering the two underlying components: annual survival and reproductive success (ARS, fig. 3, 4). Here, immigrants had similar annual adult survival probabilities to natives in both sexes (fig. 3A-E). Meanwhile, male F1s had higher survival probabilities than both immigrants and natives (fig. 3B), and hence than the immigrant-native mean ($\mu_{(I,NN)}$), such that 96% of the posterior distribution for $\Delta_{F1\text{-}\mu(I,NN)}$ exceeded zero (representing a substantial increase in survival probability of 0.16, 95%CI -0.02; 0.33, fig. 3D). Such positive F1 effects were less evident in females (fig. 3A,C). But, female F2s had noticeably low survival probabilities (fig. 3A), resulting in a posterior distribution of $\Delta_{F2\text{-}\mu(F1,\mu(I,NN))}$ that was entirely below zero, with a huge estimated decrease in survival probability of -0.51 (95%CI -0.67; -0.27) (fig. 3C). Survival of F2s was lower in females than in males (fig. 3B,D), generating a posterior distribution for the sex difference that was entirely below zero (fig. 3E). Finally, backcrosses tended to have lower annual survival probability than the F1-native mean ($\mu_{(F1,NN)}$) in both sexes; 70% and 80% of the posterior distributions of $\Delta_{Bc\text{-}\mu(F1,NN)}$ were below zero in females and males respectively.

Immigrants had similar ARS to natives in both sexes (fig. 4A,B), and posterior distributions of the difference ($\Delta_{I\text{-}NN}$) substantially overlapped zero (fig. 4C,D). Yet, $\Delta_{I\text{-}NN}$ tended to be greater in females than males (fig. 4E). Meanwhile, male F1s had higher ARS than immigrants and natives (fig. 4B), with 99% of the posterior distribution of $\Delta_{F1\text{-}\mu(I,NN)}$ exceeding zero (Figure 4D), but there were no such effects in females (fig. 4A,C). Male F2s had low ARS compared to the other groups (fig. 4B), such that the posterior distribution for $\Delta_{F2\text{-}\mu(F1,\mu(I,NN))}$ was entirely below zero, translating to -1.9 (95%CI -3.1; -0.7) fewer offspring produced by F2s per year (fig. 4E). Unexpectedly, female F2s had noticeably high ARS compared to immigrants, natives and F1s (approximately double that of natives, fig. 4A). Accordingly, 95% of the posterior distribution for $\Delta_{F2-\mu(F1,\mu(I,NN))}$ exceeded zero, translating to 3.9 (95%CI -0.3; 11.2) more offspring produced (fig. 4C), despite the small sample size of F2 females and associated uncertainty. Consequently, $\Delta_{F2-\mu(F1,\mu(I,NN))}$ differed substantially between the sexes (fig. 4E). The backcrosses showed qualitatively similar patterns, such that 94% of the posterior distribution of $\Delta_{Bc-\mu(F1,NN)}$ for males was below zero, and 64% of the posterior distribution for females exceeded zero (fig. 4C,D), resulting in a sex difference for which 94% of the posterior distribution exceeded zero (fig. 4E).

Hence overall, in males, the high adult LRS in F1s and low LRS in F2s (fig. 2B,D) were jointly caused by synergistic effects through both annual survival and ARS (fig. 3B,D, 4B,D). Meanwhile, in females, the relatively weak overall effects on LRS (fig. 2,A,C) obscured strong opposing underlying effects on fitness components in F2s, where very low annual survival (fig. 3A,C) was counteracted by very high ARS (fig. 4A,C).

Juvenile survival

Overall, there was less evidence that juvenile survival differed between the focal groups of individuals. Juvenile survival probabilities did not differ markedly between F1s and natives in either sex (Fig.5 A,B, C,D). Female F2s tended to have lower juvenile survival probability than the other groups, and hence than the F1-native mean ($\mu_{(F1,NN)}$, fig. 5A, C). However, there was little evidence of similar effects in males (fig. 5D), although the substantial uncertainty resulted in only weak evidence of a sex-specific effect (fig. 5E). Juvenile survival probabilities of backcrosses were also similar to the F1s and natives in both sexes, meaning that posterior distributions for $\Delta_{Bc\text{-}\mu(F1,NN)}$ were centered on zero (fig. 5C,D,E).

Zygote survival

Zygote survival probability differed substantially between the focal groups (with both sexes combined). Survival probabilities of F1 zygotes were 0.21 (95%CI 0.06; 0.35) higher than the native-native group zygotes (fig. 6A), such that 99.5% of the posterior distribution of $\Delta_{F1\text{-}NN}$ exceeded zero (Fig. 6B). Meanwhile, F2 zygotes had notably lower survival probabilities than other groups, up to approximately 0.20 lower than for F1s (fig. 6A). Hence, 93% of the posterior distribution of $\Delta_{F2\text{-}\mu(F1,NN)}$ was less than zero, despite considerable uncertainty (fig. 6B). Survival probabilities of backcross zygotes were close to those for F1s and hence higher than for the native-native group (fig. 6A). Thus, 89% of the posterior distribution for $\Delta_{Bc\text{-}\mu(F1,NN)}$ exceeded zero (fig. 6B). Hence, in contrast to all other fitness components, the comparisons $\Delta_{F2\text{-}\mu(F1,NN)}$ and $\Delta_{Bc\text{-}\mu(F1,NN)}$ for zygote survival showed strong effects in opposite directions (fig. 6B).

Additional analyses

Key conclusions regarding the relative values of F1 and F2 versus parental fitness components, and resulting evidence of F1 heterosis and F2 epistatic breakdown, were robust to projected values for immigrants. Specifically, observed F1 and F2 values require biologically implausible values for immigrants in the absence of dominance and epistasis, such as survival probabilities approaching zero or one, and unfeasibly high reproductive success (Supporting Information S7).

Conclusions regarding differences among focal groups also remained qualitatively similar when models were fitted including regressions of fitness components on *f*. However, estimated effect sizes for native male LRS, ARS, and juvenile survival were somewhat smaller, as expected given known inbreeding depression in these fitness components (Supporting Information S8).

Overall fitness approximation

Our measure of overall fitness, comprising the product of zygote and juvenile survival probabilities and adult LRS, hence effectively representing the number of (~6 day old) banded offspring produced by each zygote, revealed the expected net outcome of diverse effects acting across the underlying fitness components. Male F1s had overall fitness that was approximately three times greater than the native-native group (fig. 7B), translating to 1.5 more banded offspring produced by each zygote (95%CI 0.1; 4.0). However, there was little difference between F1s and natives in females (fig. 7A). Hence, 98% and 62% of the posterior distributions of $\Delta_{F1\text{-}NN}$ exceeded zero respectively (fig. 7C, D). There was a strong sex difference, with 94% of the posterior distribution below zero (fig 7E). Meanwhile, F2s tended to have lower fitness than the native-native group and the F1s in both sexes (fig. 7A,B), with 93% and 95% of the posterior distributions of $\Delta_{F2\text{-}\mu(F1,NN)}$ below zero (fig. 7C,D). Hence, F2 zygotes on average produced -0.5 (95%CI -1.2; 0.3) and -0.8 (95% CI -2.1; 0.2) less banded offspring than the F1-native mean (μ(F1,NN)) in females and males respectively. Backcrosses had lower fitness than $\mu_{(F1,NN)}$ in males, with 79% of the posterior distribution of $\Delta_{Bc-\mu(F1,NN)}$ below zero, but not in females (fig 7C,D). Yet, there was no strong evidence of sex differences in the F2 and backcross comparisons (fig. 7E).

These overall results demonstrate relatively high and low overall fitness in F1s and F2s respectively, with backcrosses tending in the same overall direction as the F2s. These conclusions remained broadly similar assuming constant juvenile survival across groups (Supporting Information S6). However, invoking known low immigrant additive genetic values for immigrants on local juvenile survival generated increased evidence of high F1 fitness in males (Supporting Information S6).

Discussion

Quantifying fitness of natural immigrants and their descendants can reveal underlying non-additive genetic effects, allowing broad inference on genetic architectures underlying sub-population divergence, and ultimately illuminating effective rates of gene flow and generational time-courses of introgression (Ingvarsson and Whitlock 2000; Lenormand 2002; Tallmon et al., 2004; Rhode and Cruzan 2005; Hansen 2013, Grummer et al., 2022). Our analyses of multi-generational data from free-living song sparrows revealed substantial fitness differences among focal filial groups, consistent with strong positive heterosis followed by severe epistatic breakdown, with notable differences between males and females and among fitness components. These results highlight that striking and multi-faceted non-additive genetic effects on fitness can arise within naturally structured meta-population systems experiencing regular internal immigration.

Evidence of heterosis

F1 offspring of immigrant-native pairings had notably high fitness components compared to the means across one or both parental groups, especially for zygote survival and adult male LRS and its sub-components annual survival and ARS. These patterns are consistent with strong heterosis, and are robust to any biologically plausible values of unobservable pre-recruitment fitness components for immigrants. Strong positive heterosis is commonly observed following crosses between highly inbred experimental and domesticated lines and remnant populations, as expected when outbreeding relieves substantial inbreeding and strong resulting inbreeding depression or drift load (Mather and Jinks, 1977; Charlesworth and Willis, 2009; Monson and Sadler 2010; Fu et al., 2014; Frankham, 2016). Yet, while it is known to experience drift, local inbreeding and resulting inbreeding depression (Wilson et al. 2011; Reid et al. 2014; Nietlisbach et al., 2017; Wolak et al., 2018), Mandarte's song sparrow population is not a highly inbred line: it lies within an interconnected meta-population and receives immigrants at rates which should in broad principle generate minimal divergence (>1 immigrant per generation, given the mean generation time of ~2.5 years, Reid et al. 2019) and replenish genetic variation (Keller et al., 2001). It is consequently notable that the magnitude of heterosis observed in zygote survival and male LRS is similar to that sometimes generated by crossing inbred lines (e.g. Monson and Sadler, 2010; Fu et al., 2014), and exceeds previously reported meta-analytic fitness increases resulting from inter-population crosses (Whitlock et al., 2013).

Such strong heterosis in spatially structured populations has been predicted theoretically. Here, mutation-selection-drift-migration balances result in accumulation of different recessive detrimental alleles in different sub-populations, which are substantially masked in F1 offspring of crosses due to increased heterozygosity. Such heterosis is predicted to be substantial given small effective population size, intermediate selection coefficients against detrimental alleles, and relatively low effective immigration rate, but estimates from natural systems that meet these criteria have been lacking (Ingvarsson and Whitlock 2000; Whitlock et al., 2000; Edmands 2007; Whitlock et al., 2013; Charlesworth 2018). Our song sparrow population likely falls broadly within such parameter space. Our results therefore support theory predicting that strong positive heterosis can arise within weakly diverged and structured natural systems.

Many experimental studies have shown that magnitudes of heterosis can vary idiosyncratically among different pairwise population crosses and environments, meaning that predictions beyond implemented crosses are challenging (e.g. Fenster and Galloway 2000; Edmands 2007; Pickup et al. 2012; Prill et al., 2014; Fitzpatrick et al., 2016). In our focal song sparrow population, immigrants are typically unrelated to each other (relative to the local pedigree baseline) and hence presumably originate from diverse or panmictic sources (Dickel et al., 2021), and fitness effects were estimated across numerous years and cohorts experiencing diverse environmental conditions (Tarwater and Arcese 2017; Tarwater, Germain and Arcese 2018). Our estimated effects can therefore be interpreted as means across multiple genetic and environmental combinations, implying that the potential for strong positive heterosis is commonplace among populations and environments within the meta-population system.

Evidence of epistatic breakdown

Multiple fitness components of F2 descendants of immigrant song sparrows were strikingly low, both in absolute terms and compared to the grand mean of the F1s and the immigrant-native or native mean, implying epistatic breakdown. These effects were particularly strong for adult male LRS and ARS, and for adult female survival even though few adult female F2s were observed, which in turn substantially reflects low survival of F2 zygotes. Such negative F2 effects have previously been observed following experimental translocations (Sagvik, Uller and Olsson 2005; Monson and Sadler 2010), with a meta-analytic decrease of 8.8% compared to parental populations following inter-population crosses (Whitlock et al. 2013). Yet, in the genetic rescue literature that primarily envisages crosses into inbred remnant populations, one widespread view is that severe outbreeding depression is unlikely unless crosses involve distinct subspecies or relatively highly diverged sub-populations that have been isolated for numerous generations or originate from different ecological conditions (Tallmon et al. 2004; Frankham et al., 2011; Ralls et al., 2018, 2020; Bell et al., 2019). Our results highlight that severe decreases in multiple F2 fitness components can arise even following natural immigration among sub-populations that are naturally and regularly interlinked on ecological timeframes.

Such low F2 fitness is even more notable since the observed F2 song sparrows are likely more heterozygous than envisaged by standard two-line-cross theory. Here, F2s have two grandparents from each of two parental lines, and are expected to be half as heterozygous as F1s (Mather and Jinks, 1977; Supporting Information S1). In contrast, since immigrant song sparrows to Mandarte are typically unrelated to each other (Dickel et al. 2021), F2s can be viewed as progeny of multi-way rather than two-way crosses. The reduction in heterozygosity in F2s compared to F1s is therefore likely to be smaller than expected, implying that epistatic breakdown must be sufficiently severe to mask ongoing heterosis. Apparent magnitudes of epistatic breakdown are consequently likely to be conservative (e.g. Whitlock et al. 2000; Rhode and Cruzan 2005).

Such strong negative effects in F2s following strong F1 heterosis are hard to alternatively explain through purely environmental mechanisms, such as inter-generational parental effects or investment. This would require F1s, which have high values of all fitness components, to produce offspring with low values for multiple fitness components ranging from zygote survival through to components of adult survival and reproductive success. Such effects would also need to be specifically manifested when two F1s mate, since backcross individuals, which also have an F1 parent, do not generally show such low fitness. Instead, poor F2 performance is consistent with genetic incompatibilities arising following recombination among native and immigrant genomes (e.g. Mather and Jinks, 1977; Lynch 1991, Lynch & Walsh 1998). This in turn implies that multi-locus genetic divergence, due to local adaptation and/or drift, must exist within the range of song sparrow dispersal, and hence likely within the local meta-population area.

Strong local divergence may itself seem implausible since regular immigration can impede local adaptive divergence by counter-acting local selection and drift (Kawecki and Ebert, 2004; Lenormand, 2002; Edmands and Timmerman, 2014; Tigano and Friesen, 2016; Brady et al. 2019; but see Fitzpatrick et al. 2020). Such outcomes depend on the degree to which physical immigration translates into effective gene flow across generations, which in turn depends on heterosis and outbreeding depression (Ingvarsson and Whitlock 2000; Porter and Benkman 2017). However, local adaptation on small spatial scales is now widely reported (Richardson et al., 2014), including among five Californian subspecies of song sparrows inhabiting adjacent saline versus freshwater environments (Walsh et al., 2019), which also exist surrounding Mandarte. The potential importance of epistatic effects in driving local adaptation has also been highlighted theoretically (Hansen 2013). Full genome analyses of immigrants and their descendants may now be useful to identify chromosome regions or gene complexes which underlie unobserved non-additive effects and population divergence (Bell et al., 2019).

Sex-specific and juvenile effects

Sex-specific differences in fitness components between immigrants and natives have been widely predicted, and observed in diverse vertebrate taxa, where immigrants of the more dispersive sex often have higher fitness on average (e.g. Hansson, Bensch, and Hasselquist, 2004; Barbraud and Delord, 2020; Martining et al., 2020; reviewed in Li and Kokko, 2019). In song sparrows, male immigrants tended to have lower LRS than natives, with no clear difference in females (which comprised 63% of immigrants). In contrast, there were clear sex differences in adult fitness components in subsequent generations. Specifically, F1 heterosis in ARS was greater in males than females, while F2 epistatic breakdown in adult annual survival was greater in females than males.

Strong heterosis in male ARS is consistent with previous estimates of very strong inbreeding depression, resulting partly from inbreeding depression in extra-pair reproduction (Reid et al. 2014, 2018, Nietlisbach et al., 2017, Wolak et al. 2018). Meanwhile, low survival of female F2s could be viewed as broadly consistent with Haldane’s rule, which predicts that the heterogametic sex will have lowest survival following hybridization (Haldane 1922; F1s and F2s, Delph and Demuth, 2016; F1s, Ottenburghs, 2022). Sex-specific epistatic breakdown could be shaped by sex-specific recombination, but there is as yet little evidence of such effects in birds (Sardell and Kirkpatrick 2020). Indeed, there was no evidence of dramatic sex-specific differences in zygote survival (Supporting Information S4). Further, extremely low annual survival of female F2s was counteracted by high ARS; effects on both fitness components were strongly statistically supported even though few female F2s survived to adulthood, potentially reflecting a strong reproduction-survival trade-off (Tarwater and Arcese 2017b). Consequently, there was evidence of sex-specific heterosis in LRS and overall fitness, with stronger effects in males, but little evidence of sex-specific epistatic breakdown. These results underline the importance of evaluating separate and combined sex-specific effects across all fitness components to infer net effects (Kawecki and Ebert 2004). Since reproductive success of male immigrants was relatively low, there were too few data to examine whether fitness of F1 offspring depended on the sex of their immigrant parent; such effects could further shape the net degree of sex-specific genetic introgression and associated local adaptation and evolution of dispersal (Li and Kokko, 2019).

Compared to the strong patterns evident in other fitness components, there was no immediately clear evidence of F1 heterosis or F2 breakdown in juvenile survival in either sex. However, such effects could be obscured by other simultaneously acting processes. Specifically, emigration (which is not distinguishable from mortality) could plausibly be more prevalent in F1 and F2 descendants of immigrants (e.g. Doligez and Pärt 2008; Edelaar and Bolnick, 2012; Saastamoinen et al., 2018). Indeed, immigrant song sparrows import low breeding values for local juvenile survival, as estimated using ‘genetic group animal models’ (Wolak et al. 2018; Reid et al. 2021). Further analyses that incorporated these low immigrant additive genetic values indicated that there could in fact be some F1 heterosis in juvenile survival, with little evidence of subsequent epistatic breakdown (Supporting Information S6). Our approach therefore highlights how evidence of obscured heterosis for key fitness components can be revealed using knowledge of additive effects.

Implications and prospects

Our evidence of strong heterosis and epistatic breakdown across multiple fitness components in a natural meta-population system has important implications for the ultimate impacts of immigration on recipient sub-populations, and opens multiple opportunities for future advances. Strong F1 heterosis could mean that effective rates of gene flow are substantially higher than expected simply given observed immigration rates (Ingvarsson and Whitlock 2000). Yet, such effects could be counter-acted by low F2 fitness, meaning that immigrant lineages are disproportionately lost through the second filial generation. Such dynamics could help explain the persistence of genetic divergence in a meta-population system, which presumably underlies the manifestation of both heterosis and epistatic breakdown.

Such demographic and evolutionary consequences of immigration depend not only on the magnitudes of fitness effects on specific types of descendants, but also on the frequency with which such descendants are produced, which depends on mating patterns and fitness of preceding generations (Millon et al., 2019). Specifically, even if F2 individuals have very low fitness, there will be little population-level impact if F2 individuals are rare (i.e. if F1s rarely interbreed). As might be expected (depending on relative additive, dominance and epistatic effects, fig. 1, Mather and Jinks, 1977; Lynch 1991), the estimated fitness of song sparrow backcrosses was typically higher than for F2s from the same generation. Strong negative fitness effects can thereby be avoided if F1s predominantly mate with natives, via non-random mating, or random mating in systems where immigration is infrequent. Such outcomes, which can be explicitly quantified in future, could reconcile our observations of strong individual-level epistatic breakdown occurring within a natural meta-population system with ‘genetic rescue’ predictions that population-level effects of gene flow are predominantly positive (e.g. Frankham et al., 2016; Ralls et al. 2018, 2020; but see Waller 2015), thereby providing integrated genetic and demographic understanding of ultimate impacts. Future analyses, encompassing all naturally-occurring crosses and backcrosses in our system and others, could also attempt to explicitly estimate coefficients of dominance and specific types of epistatic effects (e.g. Mather and Jinks 1977; Roff and Emerson 2006). Such ambitions now require further conceptual and analytical developments to accommodate selection and multi-way crosses, which are inevitable in wild meta-population systems.

Acknowledgments

We are deeply grateful to the Tsawout and Tseycum First Nations for allowing continuing access to X̱OX̱ DEŁ (Mandarte island) since 1957, to everyone that contributed to long-term data collection, and to the Natural Sciences and Engineering Research Council of Canada (NSERC), Hesse family, Forest Renewal Chair in Conservation Biology, Swiss National Science Foundation (recently P400PB-180870), Research Council of Norway (SFF- III, project 223257) and Norwegian University of Science and Technology (NTNU) for funding.

Author contributions

Lisa Dickel and Jane M. Reid designed and performed the analyses and drafted the manuscript. Peter Arcese undertook and oversaw data collection to create and maintain the pedigree. Pirmin Nietlisbach and Lukas F. Keller led genotyping and reconstruction of the genetic pedigree. Pirmin Nietlisbach, Lukas F. Keller and Jane M. Reid contributed to fieldwork. Debora Goedert contributed to conceptual synthesis. All authors contributed substantially to conceptual development and manuscript editing.

Data and Code Accessibility

Data and R code are available from the Dryad digital repository at https://doi.org/10.5061/dryad.fj6q57417; Dickel et al., 2023).

Literature cited

Åkesson, M., Liberg, O., Sand, H., Wabakken, P., Bensch, S., Flagstad, Ø. 2016. Genetic rescue in a severely inbred wolf population. Molecular Ecology 25:4745-4756.

Almeida, R. A., P. Lemmens, L. De Meester, and K. I. Brans. 2021. Differential local genetic adaptation to pesticide use in organic and conventional agriculture in an aquatic non-target species. Proceedings of the Royal Society B 288:20211903.

Arcese, P., J. N. M. Smith, and W. M. Hochachka. 1992. Stability, regulation, and the determination of abundance in an insular Song Sparrow population. Ecology 73: 805–822.

Armbruster, P., W. E. Bradshaw, and C. M. Holzapfel. 1997. Evolution of the genetic architecture underlying fitness in the pitcher-plant mosquito, *Wyeomyia smithii.* Evolution 51:451-458.

Atsumi, K., M. Lagisz, and S. Nakagawa. 2021. Nonadditive genetic effects induce novel phenotypic distributions in male mating traits of F1 hybrids. Evolution 75: 1304–1315.

Barbraud, C., and K. Delord. 2020. Selection against immigrants in wild seabird populations. Ecology Letters 24: 84-93.

Bell, D. A., Z. L. Robinson, W.C. Funk, S. W. Fitzpatrick, F. W. Allendorf, D. A. Tallmon, and A. R. Whiteley. 2019. The exciting potential and remaining uncertainties of genetic rescue. Trends in Ecology and Evolution 34: 1070–1079.

Brady, S. P., D. I. Bolnick, A. L. Angert, A. Gonzalez, R. D. H. Barrett, E. Crispo, A. M. Derry, C. G. Eckert, D. J. Fraser, G. F. Fussman, F. Guichard, T. Lamy, A. G. McAdam, A. E. M. Newman, A. Paccard, G. Rolshausen, A. M. Simons, A. P. Hendry. 2019. Causes of maladaptation. Evolutionary Applications 12: 1229–1242.

Charlesworth, B. 2018. Mutational load, inbreeding depression and heterosis in subdivided populations. Molecular Ecology 27: 4991–5003.

Charlesworth, D., and J. H. Willis. 2009. The genetics of inbreeding depression. Nature Reviews Genetics 10: 783–796.

Cheptou, P. O., Donohue, K. 2010. Environment-dependent inbreeding depression: its ecological and evolutionary significance. New Phytologist 189:395-407.

Crnokrak, P., and D. A. Roff. 1999. Inbreeding depression in the wild. Heredity, *83*: 260–270.

Delph, L. F., and J. P. Demuth. 2016. Haldanes Rule: Genetic bases and their empirical support. The Journal of Heredity 107:383-391.

De Villemereuil, P., H. Schielzeth, S. Nakagawa, and M. Morrissey. 2016. General methods for evolutionary quantitative genetic inference from generalized mixed models. Genetics 204: 1281–1294.

Dickel, L., P. Arcese, P. Nietlisbach, L. F. Keller, H. Jensen, and J. M. Reid. 2021. Are immigrants outbred and unrelated? Testing standard assumptions in a wild metapopulation. Molecular Ecology 30: 5674–5686.

Dickel, L., P. Arcese, L. F. Keller, P. Nietlisbach, D. Goedert, H. Jensen, and J. M. Reid. 2023. Data for: Multi-generational fitness effects of natural immigration indicate strong heterosis and epistatic breakdown in a wild bird population. [Dataset]. Dryad. https://doi.org/10.5061/dryad.fj6q57417

Doligez, B., and T. Pärt. 2008. Estimating fitness consequences of dispersal: A road to “know-where”? Non-random dispersal and the underestimation of dispersers’ fitness. Journal of Animal Ecology 77: 1199–1211.

Edelaar, P., and D. I. Bolnick. 2012. Non-random gene flow: An underappreciated force in evolution and ecology. Trends in Ecology and Evolution 27:659–665.

Edmands, S. 2007. Between a rock and a hard place: Evaluating the relative risks of inbreeding and outbreeding for conservation and management. Molecular Ecology 16: 463–475.

Edmands, S. and C. C. Timmerman. 2014. Modeling factors affecting the severity of outbreeding depression. Conservation Biology 17: 883-892.

Fenster, C. B. and L. F. Galloway. 2000. Population differentiation in an annual legume: genetic architecture. Evolution 54: 1157-1172.

Fitzpatrick, S. W., G. S. Bradburd, C. T. Kremer, P. E. Salerno L. M. Angeloni and W. C. Funk. 2020. Genomic and fitness consequences of genetic rescue in wild populations. Current Biology 30: 517-522.

Fitzpatrick, S. W., J. C. Gerberich; L. M. Angeloni, L. L. Bailey, E. D. Broder, J. Torres-Dowdall, C. A. Handelsman; A. López-Sepulcre, D. N. Reznick, C. K. Ghalambor and W. C. Funk. 2016. Gene flow from an adaptively divergent source causes rescue through genetic and demographic factors in two wild populations of Trinidadian guppies. Evolutionary Applications 9:879-891.

Fox, C. W., and D. H. Reed. 2011. Inbreeding depression increases with environmental stress: An experimental study and meta-analysis. Evolution 65: 246–258.

Frankham, R. 2015. Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. Molecular Ecology 24:2610–2618.

Frankham, R. 2016. Genetic rescue benefits persist to at least the F3 generation, based on a meta-analysis. Biological Conservation 195: 33–36.

Frankham, R., J. D. Ballou, M. D. B. Eldridge, R. C. Lacy, K. Ralls, M. R. Dudash, and C. B. Fenster. 2011. Predicting the Probability of Outbreeding Depression. Conservation Biology *25*: 465–475.

Fraser, D. J., L. Walker, M. C. Yates, K. Marin, J. L. A. Wood, T. A. Bernos, and C. Zastavniouk. 2019. Population correlates of rapid captive-induced maladaptation in a wild fish. Evolutionary Applications 12: 1305–1317.

Fu, D., M. Xiao, A. Hayward, Y. Fu, G. Liu, G. Jiang, and H. Zhang. 2014. Utilization of crop heterosis: A review. Euphytica 197: 161–173.

Germain, M., Pärt, T., Gustafsson, L., Doligez, B. 2017. Natal dispersers pay a lifetime cost to increased reproductive effort in a wild bird population. Proceedings of the Royal Society B 284:20162445.

Gow, E. A., P. Arcese, D. Dagenais, R. J. Sardell, S. Wilson, and J. M. Reid. 2019. Testing predictions of inclusive fitness theory in inbreeding relatives with biparental care. Proceedings of the Royal Society B 286:20191933.

Grummer, J. A., T. R. Booker, R. Matthey-Doret, P. Nietlisbach, A. T. Thomaz, and M. C. Whitlock. 2022. The immediate costs and long-term benefits of assisted gene flow in large populations. Conservation Biology 36: 1–11.

Guillaume, F., and N. Perrin. 2006. Joint evolution of dispersal and inbreeding load. Genetics 173: 497–509.

Haddad, N. M., L. A. Brudvig, J. Clobert, K. F. Davies, A. Gonzalez, R. D. Holt, T. E. Lovejoy, J. O. Sexton, M. P. Austin, C. D. Collins, W. M. Cook, E. I. Damschen, R. M. Ewers, B. L. Foster, C. N. Jenkins, A. J. King, W. F. Laurance, D. J. Levey, C. R. Margules, B. A. Melbourne, A. O. Nicholls, J. L. Orrock, D-X. Song, J. R. Townshend. 2015. Habitat fragmentation and its lasting impact on Earth's ecosystems. Science Advances 1: e1500052.

Hadfield, J. D. 2010. MCMCglmm: MCMC Methods for Multi-Response GLMMs in R. Journal of Statistical Software 33: 1–22.

Haldane, J. B. S. 1922. Sex ratio and unisexual sterility in hybrid animals. Journal of Genetics. 12: 101-109.

Hansen, T. F. 2013. Why epistasis is important for selection and adaptation. Evolution, 67: 3501–3511.

Hansson, B., S. Bensch, and D. Hasselquist. 2004. Lifetime fitness of short- and long-distance dispersing great reed warblers. Evolution 58: 2456-2557.

Hasselgren, M., A. Angerbjörn, N. E. Eide, R. Erlandsson, Ø. Flagstad, A. Landa, … K. Norén,. 2018. Genetic rescue in an inbred Arctic fox (*Vulpes lagopus*) population. Proceedings of the Royal Society B 285: 20172814.

Hastings, A., and S. Harrison. 1994. Metapopulation Dynamics and Genetics. Annual Review of Ecology and Systematics 25: 167–188.

Ingvarsson, P. K., and M. C. Whitlock. 2000. Heterosis increases the effective migration rate. Proceedings of the Royal Society B 267:1321–1326.

Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. Ecology Letters 7: 1225–1241.

Kay, M. 2021. tidybayes: Tidy Data and Geoms for Bayesian Models. doi: 10.5281/zenodo.1308151

Keller, L. F. 1998. Inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). Evolution 52: 240–250.

Keller, F., Jeffery, K. J., Arcese, P., Beaumont, M. A., Hochachka, W. M., Smith, J. N. M., Bruford, M. W. 2001. Immigration and the ephemerality of a natural population bottleneck: evidence from molecular markers. Proceedings of the Royal Society B 268: 1387-1394.

Keller, L. F., J. M. Reid and P. Arcese. 2008. Testing evolutionary models of senescence in a natural population: age and inbreeding effects on fitness components in song sparrows. Proceedings of the Royal Society B 275: 597–604.

Labroo, M. R., A. J. Studer, and J. E. Rutkoski. 2021. Heterosis and Hybrid Crop Breeding: A Multidisciplinary Review. Frontiers in Genetics 12: 643761.

Lawson Handley, L. J., and N. Perrin. 2007. Advances in our understanding of mammalian sex-biased dispersal. Molecular Ecology 16:1559–1578.

Lenormand, T. 2002. Gene flow and the limits to natural selection. Trends in Ecology and Evolution 17: 183–189.

Li, X. Y., and H. Kokko. 2019. Sex-biased dispersal: a review of the theory. Biological Reviews 94: 721–736.

Lynch, M. 1991. The Genetic interpretation of inbreeding depression and outbreeding depression. Evolution 45: 622-629.

Lynch, M., and B. Walsh. 1998. Genetics and analyses of quantitative traits. Sinauer Associates, Inc., Sunderland.

Marr, A. B., L. F. Keller, and P. Arcese. 2002. Heterosis and outbreeding depression in descendants of natural immigrants to an inbred population of song sparrows (*Melospiza melodia*). Evolution 56: 131–142.

Martinig, A. R., A. G. McAdam, B. Dantzer, J. E. Lane, D. W. Coltman, and S. Boutin. 2020. The new kid on the block: immigrant males win big whereas females pay fitness cost after dispersal. Ecology Letters 23: 430–438.

Mather, K., and J. I. Jinks. 1977. Introduction to Biometrical Genetics. Chapman and Hall, London.

Millon, A., X. Lambin, S. Devillard, and M. Schaub. 2019. Quantifying the contribution of immigration to population dynamics: a review of methods, evidence and perspectives in birds and mammals. Biological Reviews 94:2049–2067.

Mobley, K. B., H. Granroth- Wilding, M. Ellmen, J.-P. Vähä, T. Aykanat, S. E. Johnston, P. Orell, J. Erkinaro and C. R. Primmer. 2019. Home ground advantage: Local Atlantic salmon have higher reproductive fitness than dispersers in the wild. Science advances 5:eaav1112

Monson, C. A., and K. C. Sadler. 2010. Inbreeding depression and outbreeding depression are evident in wild-type zebrafish lines. Zebrafish *7:* 189–197.

Nietlisbach, P., G. Camenisch, T. Bucher, J. Slate, L. F. Keller, and E. Postma. 2015. A microsatellite-based linkage map for song sparrows (*Melospiza melodia*). Molecular Ecology Resources 15: 1486–1496.

Nietlisbach, P., L. F. Keller, G. Camenisch, F. Guillaume, P. Arcese, J. M. Reid and E. Postma. 2017. Pedigree-based inbreeding coefficient explains more variation in fitness than heterozygosity at 160 microsatellites in a wild bird population. Proceedings of the Royal Society B 284: 20162763.

Orr, H. A. 2009. Fitness and its role in evolutionary genetics. Nature Reviews Genetics 10: 531–539.

Ottenburghs, J. 2022. Avian introgression patterns are consistent with Haldane’s rule. Journal of Heredity 113:363–370.

Pickup, M. D.L. Field, D.M. Rowell, A. G. Young. 2012. Source population characteristics affect heterosis following genetic rescue of fragmented plant populations. Proceedings of the Royal Society B 280: 20122058.

Porter, C. K. and C. W. Benkman. 2017. Assessing the potential contributions of reduced immigrant viability and fecundity to reproductive isolation. American Naturalist 189: 580-591.

Postma, E., F. Heinrich, U. Koller, R. J. Sardell, J. M. Reid, P. Arcese, and L. F. Keller. 2011. Disentangling the effect of genes, the environment and chance on sex ratio variation in a wild bird population. Proceedings of the Royal Society B 278: 2996–3002.

Prill, N., J. M. Bullock, N. M. van Dam, and R. Leimu. 2014. Loss of heterosis and family-dependent inbreeding depression in plant performance and resistance against multiple herbivores under drought stress. Journal of Ecology 102: 1497–1505.

Ralls, K., J. D. Ballou, M. R. Dudash, M. D. B. Eldridge, C. B. Fenster, R. C. Lacy, … R. Frankham. 2018. Call for a Paradigm Shift in the Genetic Management of Fragmented Populations. Conservation Letters 11: 1–6.

Ralls, K., P. Sunnucks, R. C. Lacy, and R. Frankham. 2020. Genetic rescue: A critique of the evidence supports maximizing genetic diversity rather than minimizing the introduction of putatively harmful genetic variation. Biological Conservation 251: 108784.

Reid, J. M., P. Arcese, and L. F. Keller. 2006. Intrinsic parent-offspring correlation in inbreeding level in a Song Sparrow (*Melospiza melodia*) population open to immigration. American Naturalist 168: 1–13.

Reid, J. M., P. Arcese, P. Nietlisbach, M. E. Wolak, S. Muff, L. Dickel, and L. F. Keller. 2021. Immigration counter-acts local micro-evolution of a major fitness component: Migration-selection balance in free-living song sparrows. Evolution Letters 5: 48–60.

Reid, J. M., L. F. Keller, A. B. Marr, P. Nietlisbach, R. J. Sardell, and P. Arcese. 2014. Pedigree error due to extra-pair reproduction substantially biases estimates of inbreeding depression. Evolution 68: 802–815.

Reid, J. M., P. Nietlisbach, M. E. Wolak, L. F. Keller, P. Arcese. 2019. Individuals’ expected genetic contribution to future generations, reproductive value, and short-term metrics of fitness in free-living song sparrows (*Melospiza melodia*). Evolution Letters 3: 271-285.

Rhode, J. M. and M. B. Cruzan. 2005. Contributions of heterosis and epistasis to hybrid fitness. American Naturalist 166: El24-El39.

Richardson, J. L., M. Urban; D. I. Bolnick, D. K.Skelly. 2014. Microgeographic adaptation and the spatial scale of evolution. Trends in Ecology and Evolution 29: 156-176.

Roff, D. A. and K. Emerson. 2006. Epistasis and dominance: evidence for differential effects in life-history versus morphological traits. Evolution 60: 1981-1990.

Rundle, H. D., and M. C. Whitlock. 2001. A genetic interpretation of ecologically dependent isolation. Evolution 55: 198–201.

Saastamoinen, M., G. Bocedi, J. Cote, D. Legrand, F. Guillaume, C. W. Wheat, E. A. Fronhofer, C. Garcia, R. Henry, A. Husby, M. Baguette, D. Bonte, A. Coulon, H. Kokko, E. Matthysen, K. Niitepõld, E. Nonaka, V. M. Stevens, J. M. J. Travis, K. Donohue, J. M. Bullock, M. del Mar Delgado. 2018. Genetics of dispersal. Biological Reviews 93: 574–599.

Sagvik, J., T. Uller, M. Olsson. 2005. Outbreeding depression in the common frog, *Rana temporatia.* Conservation Genetics 6:205-211.

Sardell, J. M. and M. Kirkpatrick. 2020. Sex differences in the recombination landscape. American Naturalist 195: 361-379.

Sardell, R. J., L. F. Keller, P. Arcese, T. Bucher, and J. M. Reid. 2010. Comprehensive paternity assignment: Genotype, spatial location and social status in song sparrows, *Melospiza melodia*. Molecular Ecology 19: 4352–4364.

Schneemann, H., B. De Sanctis, D. Roze, N. Bierne, J. Welch. 2020. The geometry and genetics of hybridization. Evolution 74: 1575-2590.

Smith, J. N. M., L. F. Keller, A. B. Marr, and P. Arcese. 2006. Conservation and biology of small populations: the song sparrows of Mandarte Island. Oxford University Press, Oxford.

Smith, J. N. M., M. J. Taitt, C. M. Rogers, P. Arcese, L. F. Keller, A. L. E. V. Cassidy and W. M. Hochachka. 1996. A metapopulation approach to the population biology of the Song Sparrow *Melospiza melodia*. Ibis 138: 120–128.

Svensson, E. I., D. Goedert, M. A. Gómez-Llano, F. Spagopoulou, A. Nava-Bolaños, and I. Booksmythe. 2019. Sex differences in local adaptation: What can we learn from reciprocal transplant experiments? Philosophical Transactions of the Royal Society B 373: 20170420.

Tallmon, D. A., G. Luikart, R. S. Waples. 2004. The alluring simplicity and complex reality of genetic rescue. Trends in Ecology and Evolution 19: 489-496.

Tarwater, C. E. and P. Arcese. 2017. Individual fitness and the effects of a changing climate on the cessation and length of the breeding period using a 34-year study of a temperate songbird. Global Change Biology 24:1212-1223.

Tarwater, C. E., and P. Arcese. 2017b. Young females pay higher costs of reproduction in a short-lived bird. Behavioral Ecology and Sociobiology 71: 84.

Tarwater, C. E., R. R. Germain, P. Arcese. 2018. Examination of context-dependent effects of natal traits on lifetime reproductive success using a long-term study of a temperate songbird. Ornithology 135: 609-621.

Team, R. core. 2020. R: A Language and Environment for Statistical Computing. Retrieved from https://www.r-project.org/.

Teixeira, J. C., and C. D. Huber. 2021. The inflated significance of neutral genetic diversity in conservation genetics. Proceedings of the National Academy of Sciences 118: 1–10.

Tigano, A. and V. L. Friesen. 2016. Genomics of local adaptation with gene flow. Molecular Ecology 25: 2144-2164.

Waller, D. 2015. Genetic rescue: a safe or risky bet? Molecular Ecology, 24: 2595-2597.

Walsh, J., P. M. Benham, P. E. Deane-Coe, P. Arcese, B. G. Butcher, Y. L. Chan, Z. A. Cheviron, C. S. Elphick, A. I. Kovach, B. J. Olsen, W. G. Shriver, V. L. Winder, and I. J. Lovette. 2019. Genomics of rapid ecological divergence and parallel adaptation in four tidal marsh sparrows. Evolution Letters 3: 324-388.

Waser, P. M., K. M. Nichols and J. D. Hadfield. 2013. Fitness consequences of dispersal: Is leaving home the best of a bad lot? Ecology 94: 1287-1295.

Weeks, A. R., Heinze, D., Perrin, L., Stoklosa, J., Hoffmann, A. A., Van Rooyen, A., Kelly, T., Mansergh, I. 2017. Genetic rescue increases fitness and aids rapid recovery of an endangered marsupial population. Nature Communications 8:1071.

Whitlock, M. C., P. K. Ingvarsson, and T. Hatfield. 2000. Local drift load and the heterosis of interconnected populations. Heredity 84: 452–457.

Whitlock, R., G. B. Stewart, S. J. Goodman, S. B. Piertney, R. K. Butlin, A. S. Pullin, and T. Burke. 2013. A systematic review of phenotypic responses to between-population outbreeding. Environmental Evidence 2: 13.

Wickham, H., Averick, M., Bryan, J., Chang, W., D’Agostino McGowan, L., François, R., Grolemund, G. et al. 2019. Welcome to the Tidyverse. The Journal of Open Source Software. 4: 1686.

Wilson, A. G., and P. Arcese. 2008. Influential factors for natal dispersal in an avian island metapopulation. Journal of Avian Biology, 39: 341–347.

Wilson, A. G., P. Arcese, Y. L. Chan, and M. A. Patten. 2011. Micro-spatial genetic structure in song sparrows (*Melospiza melodia*). Conservation Genetics 12: 213–222.

Wilson, S., D. R. Norris, A. G. Wilson, and P. Arcese. 2007. Breeding experience and population density affect the ability of a songbird to respond to future climate variation. Proceedings of the Royal Society B 274: 2539–2545.

Wolak, M. E., P. Arcese, L. F. Keller, P. Nietlisbach, and J. M. Reid. 2018. Sex-specific additive genetic variances and correlations for fitness in a song sparrow (*Melospiza melodia*) population subject to natural immigration and inbreeding. Evolution 72: 2057–2075.

Wolf, J. B., and M. J. Wade. 2001. On the assignment of fitness to parents and offspring: Whose fitness is it and when does it matter? Journal of Evolutionary Biology 14: 347–356.

References Cited Only in the Online Enhancements

Falconer, D. S., and T. F. C. Mackay, T. F. C. 1996. Introduction to quantitative genetics, 4th ed. Longman, Harlow.

Reid, J. M., P. Arcese, R. J. Sardell, and L. F. Keller. 2011. Additive genetic variance, heritability, and inbreeding depression in male extra-pair reproductive success. American Naturalist 177: 177–187.

Result Figures

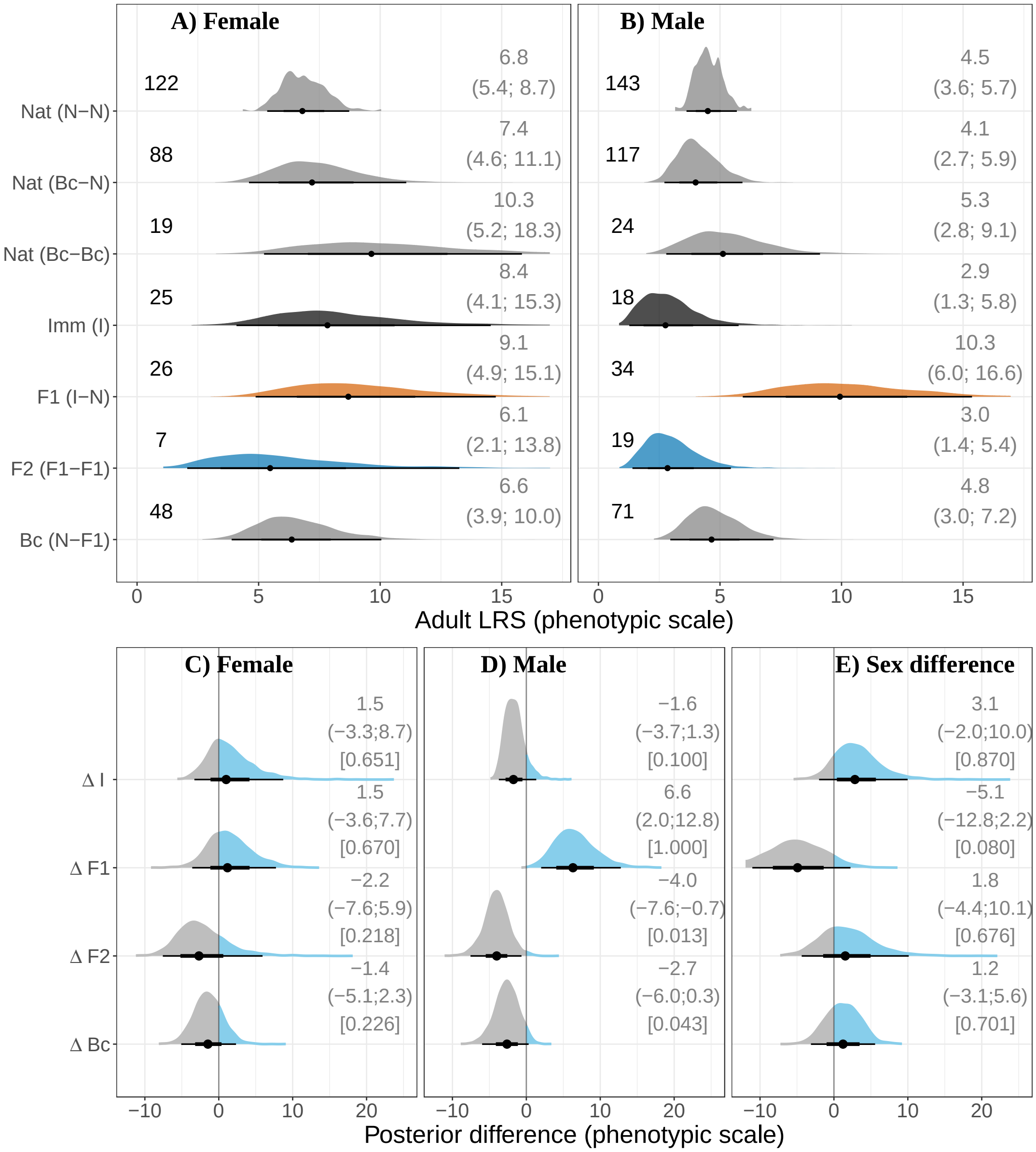


Figure 2: Posterior distributions of (A,B) adult lifetime reproductive success (LRS, total number of chicks produced) for each focal group of individuals in (A) females and (B) males, and (C,D,E) planned comparisons between groups for (C) females, (D) males and (E) the between-sex-difference. A and B show posterior distributions of adult LRS for natives divided into three sub-groups comprising individuals with eight (N-N), seven (Bc-N) or six (Bc-Bc) locally hatched great-grandparents respectively (where N and Bc denote native and backcross grandparents), immigrants, F1s, F2s, and backcrosses. Sample sizes of individuals in each group are shown in black text. C and D show posterior distributions of key differences (table 1), where ΔI represents the difference between immigrants and natives (i.e. ΔI-NN), ΔF1 the difference between F1s and μ(I,NN) (i.e. ΔF1-μ(I,NN)), ΔF2 the difference between F2s and μ(F1,μ(I,NN)) (i.e. ΔF2-μ(F1,μ(I,NN))), and ΔBc the difference between backcrosses and μ(F1,NN) (i.e. ΔBc-μ(F1,NN)). Posterior means, 95% credible intervals (CIs, in parentheses), and proportions exceeding zero for differences [in brackets] are shown in grey text. In C-E, shades highlight the proportions of the posterior distributions above versus below zero. On all panels, black points denote posterior medians, and thick and thin horizontal bars denote the 50% and 95% CIs. Shaded areas show full posterior distributions of estimated effects, not distributions of raw data. Estimates on latent and phenotypic scales, and equivalent illustrations of latent scale effects, are in Supporting Information S5 and S8, respectively.

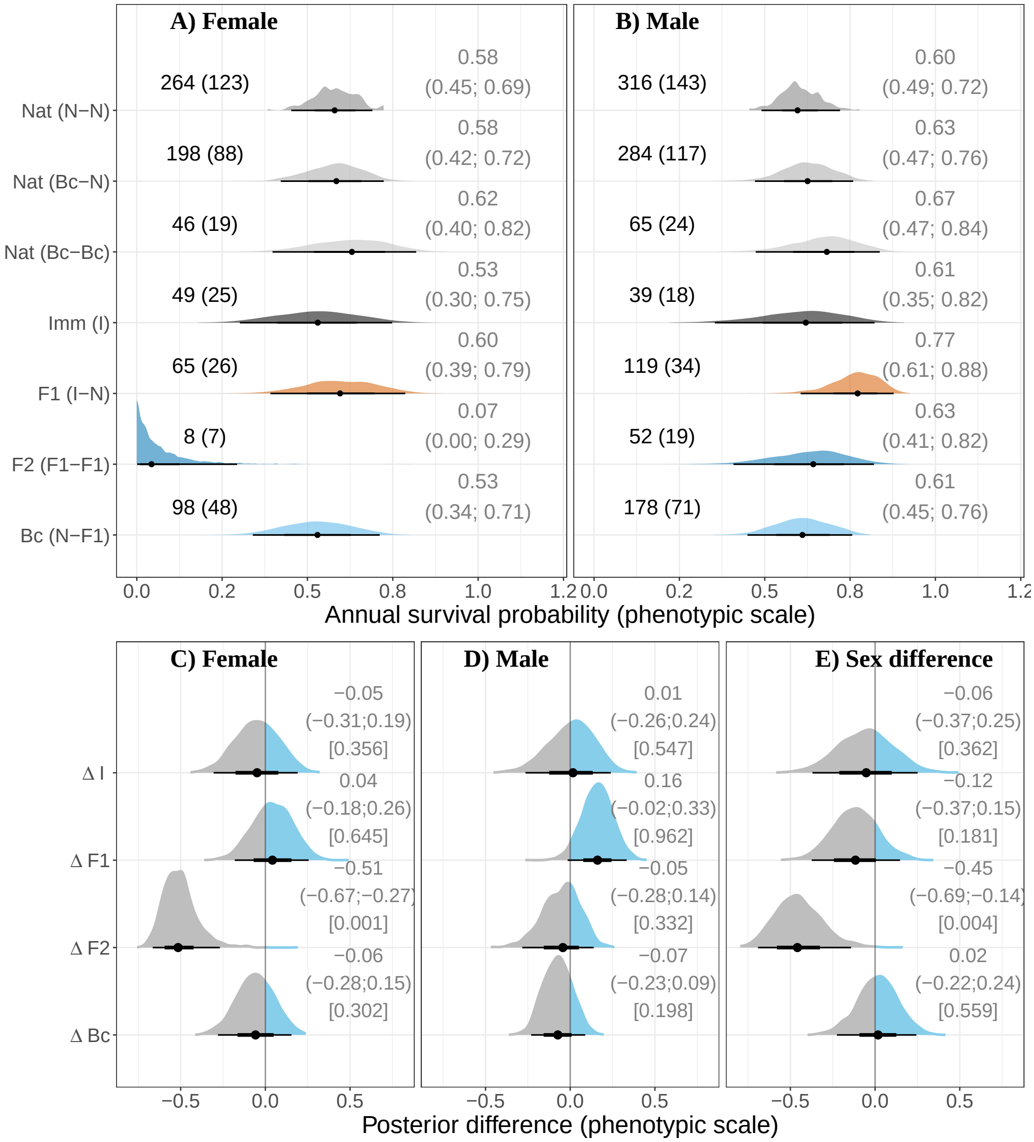


Figure 3: Posterior distributions of (A, B) annual survival probability of each focal group of individuals, and (C, D, E) planned comparisons between selected groups. Figure structure and attributes are as in Figure 2. On A and B, sample sizes of observations and individuals (in parentheses) are shown.

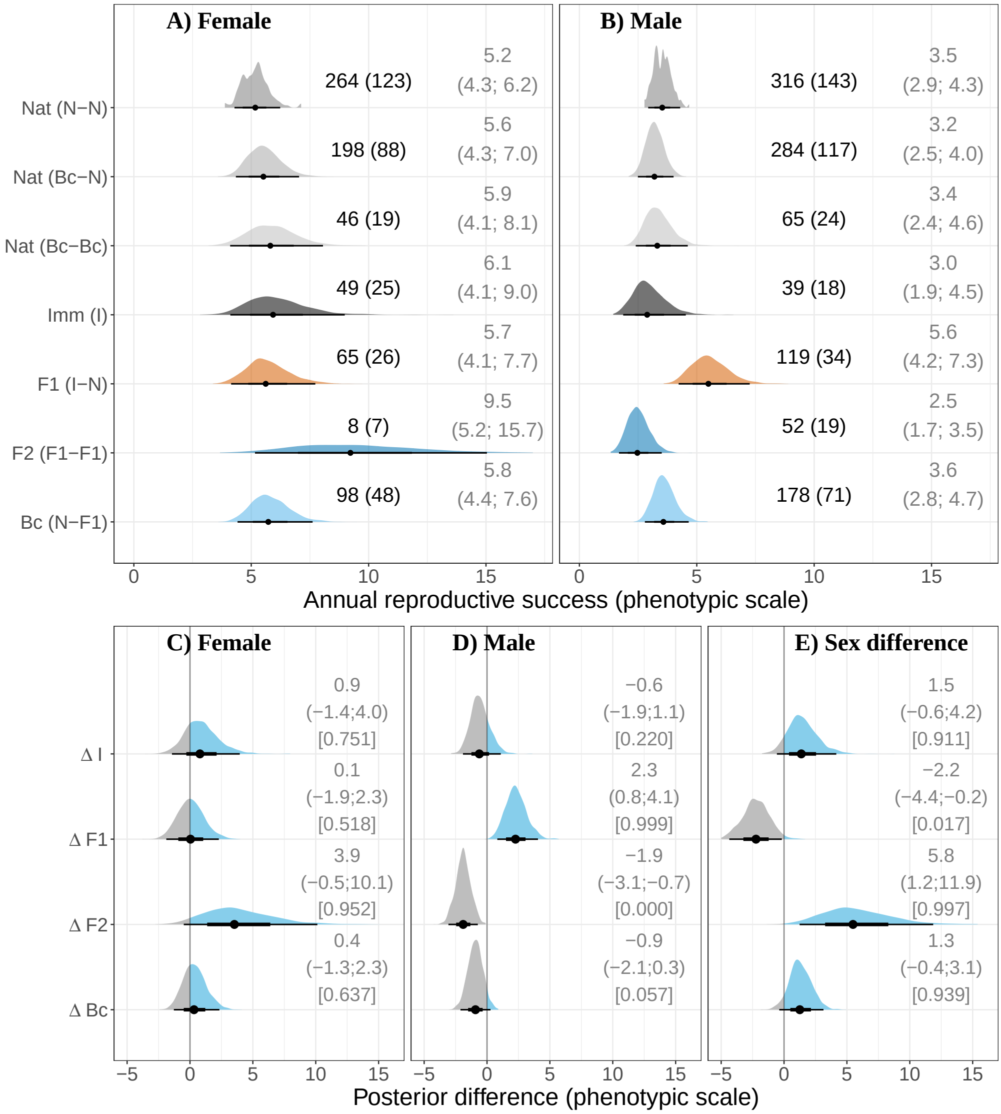


Figure 4: Posterior distributions of (A,B) annual reproductive success of each focal group of individuals and (C,D,E) planned comparisons between selected groups. Figure structure and attributes are as in Figure 2. On A and B, sample sizes of observations and individuals (in parentheses) are shown.

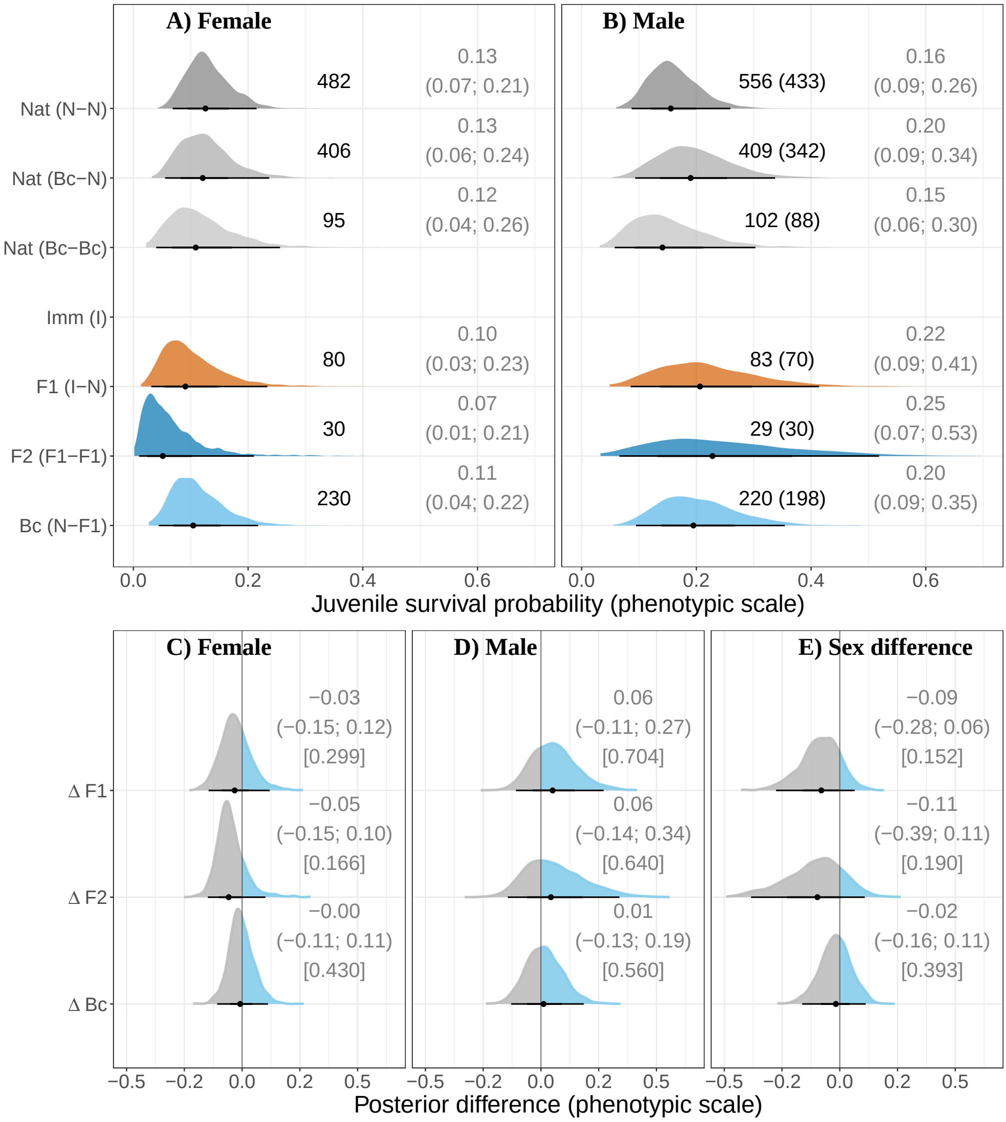


Figure 5: Posterior distributions of (A, B) juvenile survival probability for each focal group of individuals, and (C, D, E) planned comparisons between selected groups. Figure structure and attributes are the same as in Figure 2. There is no posterior distribution for immigrants because they are not present at this life stage in the local population. C and D show the posterior distributions of key differences (table 1), where ΔF1 represents the difference

between F1s and natives (i.e. ΔF1-NN), ΔF2 the difference between F2s and μ(F1,NN) (i.e. ΔF2-μ(F1,NN)) with no immigrant component, and ΔBc the difference between backcrosses and μ(F1,NN) (i.e. ΔBc-μ(F1,NN)). In A and B, sample sizes of individuals and clutches (in parentheses, collectively for males and females) are shown in black text for each group.

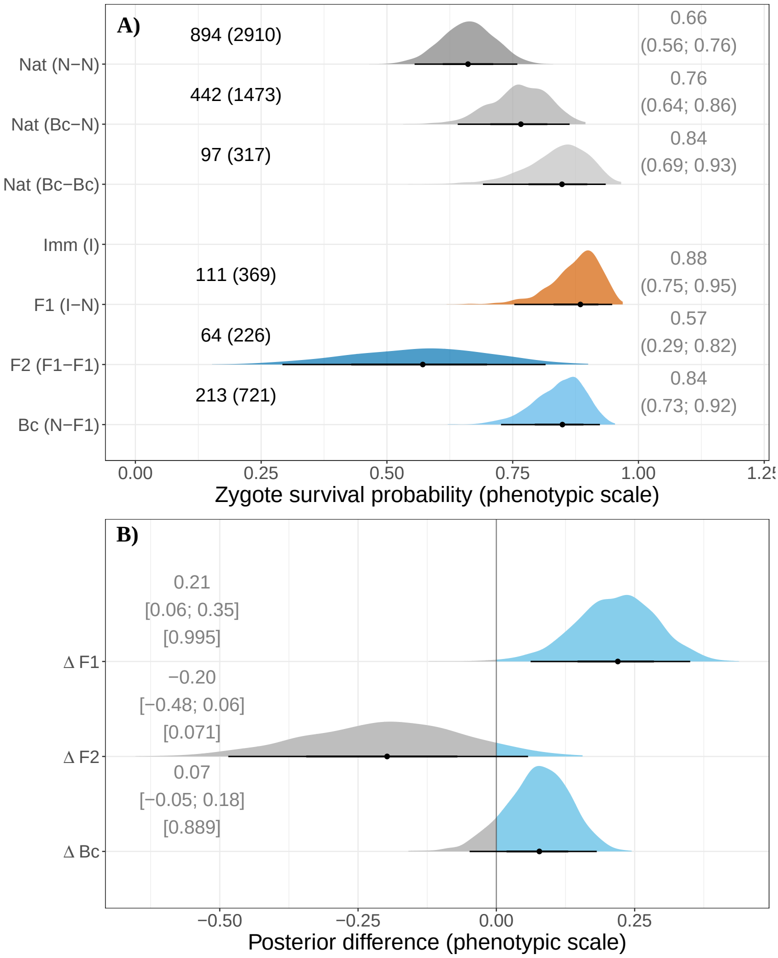


Figure 6: Posterior distributions of (A) zygote survival probability (phenotypic scale) for each focal group of clutches and (B) planned comparisons between selected groups. Figure structure and attributes are as in Figure 5 (again with no immigrants), except that females and males are combined. On A, sample sizes of clutches and individual eggs (in parentheses) are shown.

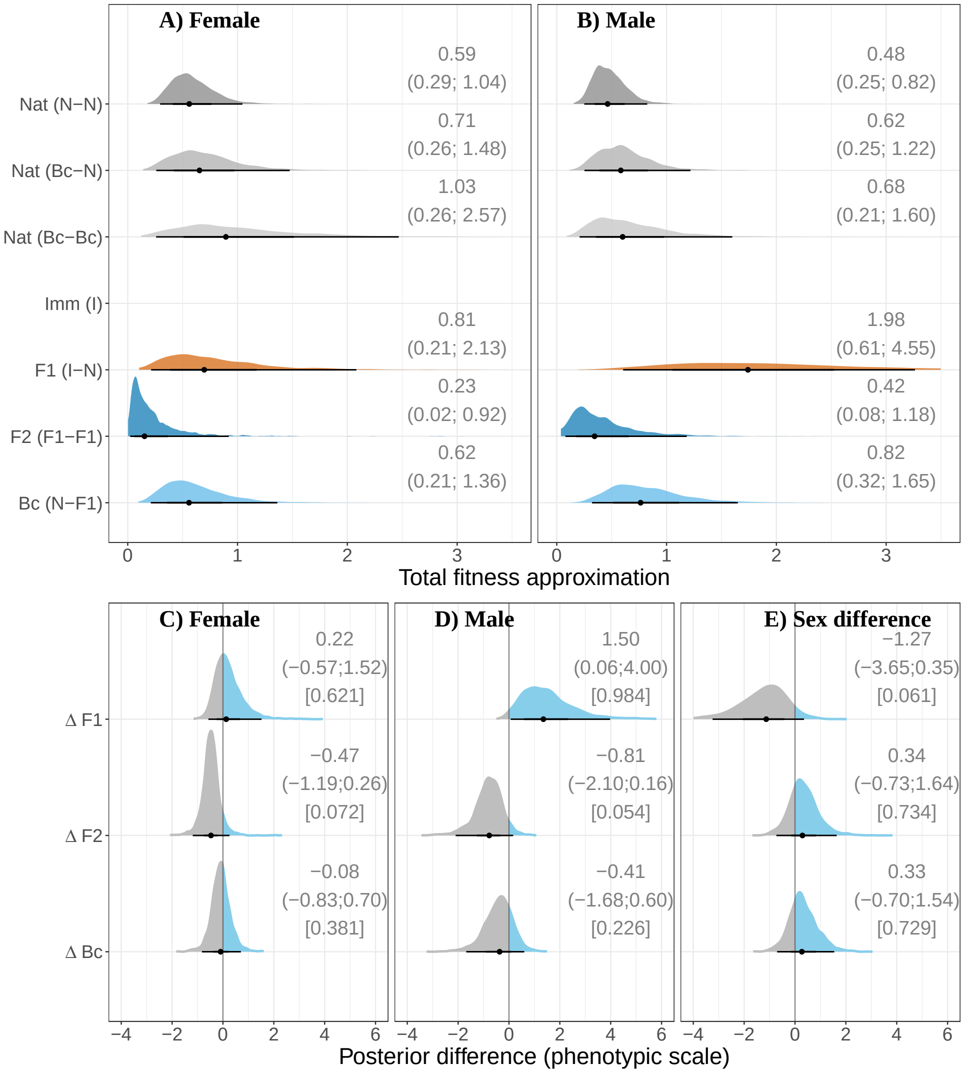


Figure 7: Posterior distributions (A,B) of overall fitness approximated as the product of zygote and juvenile survival probabilities, and adult LRS (phenotypic scale) of each focal group for females (A) and males (B) and (C,D, E) planned comparisons between selected groups for females (C), males (D), and (E) the between-sex difference. Figure structure and attributes are as in Figure 5 (again with no immigrants). No sample sizes are shown because the depicted approximations are functions of posterior distributions from analyses of different fitness components with varying sample sizes.

The American Naturalist

Supporting Information for:

Multi-generational fitness effects of natural immigration indicate strong heterosis and epistatic breakdown in a wild bird population

Lisa Dickel[1], Peter Arcese[2]**, Lukas F. Keller[3,4], Pirmin Nietlisbach[5], Debora Goedert[1], Henrik Jensen**[1]**, Jane M. Reid**[1,6]

[1]Centre for Biodiversity Dynamics, Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway

[2]Department of Forest & Conservation Sciences, University of British Columbia, Vancouver, British Columbia, Canada

[3]Department of Evolutionary Biology & Environmental Studies, University of Zurich, Zurich, Switzerland

[4]Natural History Museum, University of Zurich, Zurich, Switzerland

[5]School of Biological Sciences, Illinois State University, Normal, Illinois, USA

[6] School of Biological Sciences, University of Aberdeen, Aberdeen, UK

Corresponding author: Lisa Dickel, email: dickel.lisa@gmail.com

Table of contents

Supporting Information S1: Conceptual framework

Supporting Information S2: Group explanations

Supporting Information S3: Fitness components and comparison to Marr et al. 2002

Supporting Information S4: Data selection and checks for biases

Supporting Information S5: Models, data summaries and model coefficients

Supporting Information S6: Alternative overall fitness approximation with constant juvenile survival

Supporting Information S7: Hypothetical immigrant values

Supporting Information S8: Models accounting for pedigree *f*

Supporting Information S1: Conceptual framework

Derivations of key quantities and comparisons from line-cross theory

Established line-cross theory provides expressions for the contributions of additive, dominance and epistatic effects to the mean phenotypic values of different parental groups and their descendants (i.e. filial generations). Such contributions can be conveniently defined relative to the parental groups, or relative to the F2 population (which is expected to be in Hardy-Weinberg equilibrium given a standard two-line cross, Lynch 1991; Roff and Emerson 2006). For current purposes we define contributions relative to the parent groups, thereby envisaging the mean as the mean of all inbred lines that could in principle be generated from a cross (Roff and Emerson 2006). Accordingly,

$P_1 = \mu + A + E_{AA}$

$P_2 = \mu - A + E_{AA}$

$F1 = \mu + D + E_{DD}$

$F2 = \mu + \frac{1}{2}D + \frac{1}{4}E_{DD}$

$B_1 = \mu + \frac{1}{2}A + \frac{1}{2}D + \frac{1}{4}E_{AA} + \frac{1}{4}E_{AD} + \frac{1}{4}E_{DD}$

(following Mather and Jinks 1977 P.107; Roff and Emerson 2006 Table 1). Here, P_1 and P_2 are the two parental lines. F1 is the cross between P_1 and P_2.

F2 is the cross between two F1s. B_1 is the backcross of the F1 to P_1. Subscripts 1 and 2 denote the two lines, while F1 and F2 denote the first and second filial generations. μ denotes the mean and A, D and E denote additive, dominance and epistatic genetic effects respectively. More specifically, E_{AA}, E_{AD} and E_{DD} denote additive-by-additive, additive-by-dominance and dominance-by-dominance epistasis respectively. Higher-order forms of epistasis are effectively assumed to be negligible.

The above expressions arise because F1s are envisaged to be entirely heterozygous, in the sense of inheriting a P_1 and a P_2 allele at all autosomal loci. Dominance effects are therefore maximal. Half this heterozygosity is lost in the F2 (Falconer and Mackay 1996). An analogous expression to that for B_1 can be derived for the backcross between F1 and P_2 (i.e. B_2). However, taking P_1 as natives and P_2 as immigrants in a focal wild population, such a B_2 backcross may not be locally observed (without substantial inbreeding resulting from back-crossing of a focal F1 to its own P_2 immigrant parent).

Informative sets of means and comparisons can then be derived, taking P_1 as natives (NN) and P_2 as immigrants (I) with abbreviations defined in main manuscript table 1:

Difference between parents: $\Delta I\text{-}NN = 2A$

Parent mean: $\mu(I,NN) = ½(2\mu + 2E_{AA}) = \mu + E_{AA}$

Difference between F1 and parent mean = $\Delta F1\text{-}\mu(I,NN) = D + E_{DD} - E_{AA}$

Grand mean of F1 and parent mean: $\mu(F1,\mu(I,NN)) = \frac{1}{2}(2\mu + D + E_{DD} + E_{AA}) = \mu + \frac{1}{2}D + \frac{1}{2}E_{DD} + \frac{1}{2}E_{AA}$

Difference between F2 and the grand mean of F1 and parent mean: $\Delta F2\text{-}\mu(F1, \mu(I,NN)) =$

$-\frac{1}{4}E_{DD} - \frac{1}{2}E_{AA}$

Difference between B1 and the mean of F1 and P_1 (i.e. NN): $\Delta Bc\text{-}\mu(F1,NN) = -\frac{1}{4}E_{AA} + \frac{1}{4}E_{AD} - \frac{1}{4}E_{DD}$

Consequently, if there is no epistasis (i.e. $E_{AA} = E_{AD} = E_DD = 0$) then:

Difference between F1 and parent mean: $\Delta F1\text{-}\mu(I,NN) = D$

Difference between F2 and the grand mean of F1 and parent mean: $\Delta F2\text{-}\mu(F1,\mu(I,NN)) = 0$

Difference between B1 and the mean of F1 and P_1 (i.e. NN): $\Delta Bc\text{-}\mu(F1,NN) = 0$

Estimating the difference between the F2 and the grand mean of the F1 and parent mean ($\Delta F2\text{-}\mu(F1,\mu(I,NN))$), and estimating the difference between B1 and the mean of F1 and P_1 (i.e. NN, hence $\Delta Bc\text{-}\mu(F1,NN)$), both give composite estimates of epistatic effects on the trait mean. If these differences differ from zero, there is evidence of epistasis. The contributions of additive-by-additive, additive-by-dominance and dominance-by-dominance epistasis cannot be readily distinguished, although this could potentially be attempted in future by considering continuous variation in source and hybridity indices across all observed individuals (e.g. Lynch 1991).

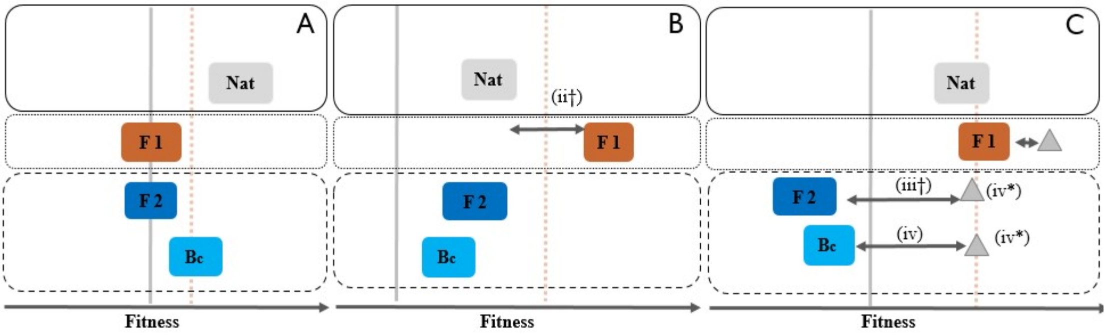
The measure $\Delta_{F2-\mu(F1,\mu(I,NN))}$ has the advantage that epistatic effects could be greater (for example involving ½E_{AA}), but the disadvantage that it requires an estimate of the mean of P_1 and P_2 (i.e.,$\mu_{(I,NN)}$), which in turn requires estimates from both parental lines. This might not be feasible, for example for early-life fitness components when P_2 is an immigrant lineage. In contrast, the measure $\Delta_{Bc-\mu(F1,NN)}$ has the advantage that it does not involve P_2, and hence can be fully quantified given data collected in a single focal population receiving immigrants. But it has the disadvantage that epistatic effects could be smaller (for example involving only ¼E_{AA}). Power to detect differences from zero, and hence reject the hypothesis of no epistasis, might therefore be lower. In the absence of epistasis, the difference between the F_1 and the parent mean ($\Delta_{F1-\mu(I,NN)}$) gives an estimate of total dominance effects on the trait mean. However, with epistasis, this value also includes non-identifiable components of additive-by-additive and dominance-by-dominance epistasis.

When the defined P_2 comprises the set of observed immigrants into a focal population, the P_2 might be relatively heterogeneous if immigrants arise from different source populations. The above expressions then still broadly hold, effectively envisaging the P_2 as a large panmictic population even though observed immigrants may originate from relatively small populations and hence be somewhat inbred. However, F2s may be more heterozygous than expected if their two immigrant grandparents originate from different source populations. In that case, estimates of epistatic breakdown inferred from $\Delta_{F2-\mu(F1,\mu(I,NN))}$ will likely be conservative. This situation also means that, for current purposes, it is most convenient to define focal quantities relative to the parental populations rather than the F2 (e.g. Lynch 1991; Roff and Emerson 2006). There are also expectations that variances will differ among groups of individuals (see Mather and Jinks, 1977; Lynch and Walsh 1998), but these expectations are not developed or tested here.

Unobservable immigrant fitness components

Because pre-dispersal fitness components were unobservable for immigrants, i.e. zygote and juvenile survival probability, and hence also the cumulative fitness approximation, we developed alternative, reduced comparisons for these planned comparisons, which are not relying on immigrant values. These are developed in fig. S1 and table 1 in the main text in order to still infer heterosis and epistatic breakdown in the absence of immigrant data.

Figure S1: Conceptual illustration of expected fitness (x-axis) of distinct groups of individuals following immigration for pre-dispersal fitness components where immigrants are unobservable. (A) Expectation given solely additive genetic effects, where additive genetic values of immigrants in the recipient population environment are unknown but assumed to be lower than natives, consistent with local adaptation. (B) Expectation given additive and dominance genetic effects, where F1 fitness deviates from the native value, here depicted as positive heterosis. (C) Expectation given additive, dominance and epistatic genetic effects, where F2 fitness deviates from the grand mean of the F1 and the native mean, and backcross fitness deviates from the F1-native mean, here depicted as epistatic breakdown. ‘Natives’ (Nat) are individuals with local ancestry; F1s are offspring of immigrant-native matings; F2s are offspring of F1-F1 matings; backcrosses (Bc) are offspring of matings between an F1 and one of the parental groups, here depicted as an F1-native mating. The y-axis dimension represents successive generations: solid boxes denote the parental generation (natives), dotted boxes denote the first filial generation (F1s), and dashed boxes denote the second descendant generation (F2s, backcrosses). Filled boxes denote defined groups of individuals, where colors match Results figures. On B and C, circles and triangles denote expectations given solely additive, or additive and dominance genetic effects, respectively. Arrows denote deviations from these expectations, given (B) dominance and (C) dominance and epistasis. Solid and dashed vertical lines indicate the (hypothetical) immigrant-native and native-F1 means respectively. Roman numerals link to planned comparisons listed in table 1 in the main text, where asterisks (*) denote means across groups, and '†' denotes comparisons for pre-dispersal fitness components.



Supporting Information S2: Group explanations

To compute the planned comparisons and thereby test for evidence of heterosis and epistasis, we grouped individuals depending on their ancestry. The different groups are defined here, where individuals that hatched within the focal study population on Mandarte are denoted by "Locally hatched ("LH"), and individuals that immigrated are denoted by "Imm". Figure S2.1 shows the defined natives, encompassing the three sub-groups of native-native, native-backcross and backcross-backcross. Figure S2.2 shows the defined F1s, F2s and Backcrosses. Individuals with ancestries that were not of direct current interest for testing hypotheses were pooled into an "other" group (summarized in table S2.1). When comparing fitness components, e.g. F1s to the mean of natives and immigrants, we compare them to overall group means rather than their specific parents, thereby estimating overall mean effects rather than individual immigrants' effects.

Figure S2.1: Illustration of ancestry of three different native groups, (A) Native-natives (N-N) with eight locally hatched (LH) great-grandparents, (B) Backcross- natives (Bc-N) with seven locally hatched great-grandparents and one immigrant great-grandparent, and (C) Backcross-backcrosses (Bc-Bc) with six locally hatched great-grandparents. Groups of individuals which locally hatched (LH) individuals can belong to are indicated in the figure, e.g. as "LH / F1" for a locally hatched F1 individual. We excluded individuals that had F2 parents as 'Natives' (they would be F3s if they had two F2 parents). This results in an exclusion criteria, that if a focal individual has less than six locally hatched great-grandparents, it is no longer defined as a 'Native'. Two immigrant great-grandparents cannot be in the ancestry of one grandparent of the focal 'Native' individual, otherwise the focal individual would be an F1 or Backcross (fig. S2.2).

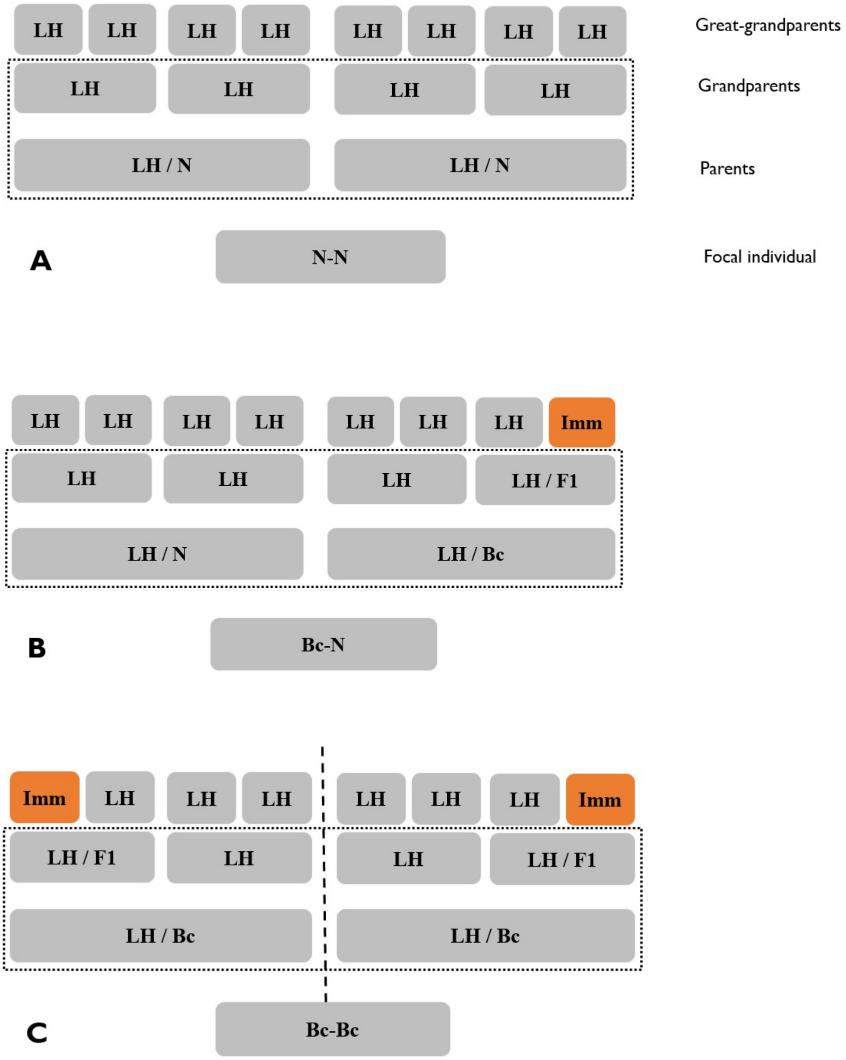

LH
LH
LH
LH
LH
LH
LH
LH
Great-grandparents
LH
LH
LH
LH
Grandparents
LH / N
LH / N
Parents
A
N-N
Focal individual
LH
LH
LH
LH
LH
LH
LH
Imm
LH
LH
LH
LH / F1
LH / N
LH / Bc
B
Bc-N
Imm
LH
LH
LH
LH
LH
LH
Imm
LH / F1
LH
LH
LH / F1
LH / Bc
LH / Bc
C
Bc-Bc

Figure S2.2: Illustration of ancestry of (A) F1 offspring of immigrant-native pairings, (B) F2 offspring of F1-F1 pairings, and (C) Backcross offspring of F1-native pairings. Specifically, F1s have one locally hatched parent with four locally hatched grandparents (which can be N-N, Bc-N or Bc-Bc, see Fig.2.1), and one immigrant parent (i.e. not hatched on Mandarte). F2s have two F1 parents. Here, F2s might effectively represent crosses of three rather than two lines, if their two immigrant grandparents are unrelated. Backcrosses have one defined native parent and one F1 parent.

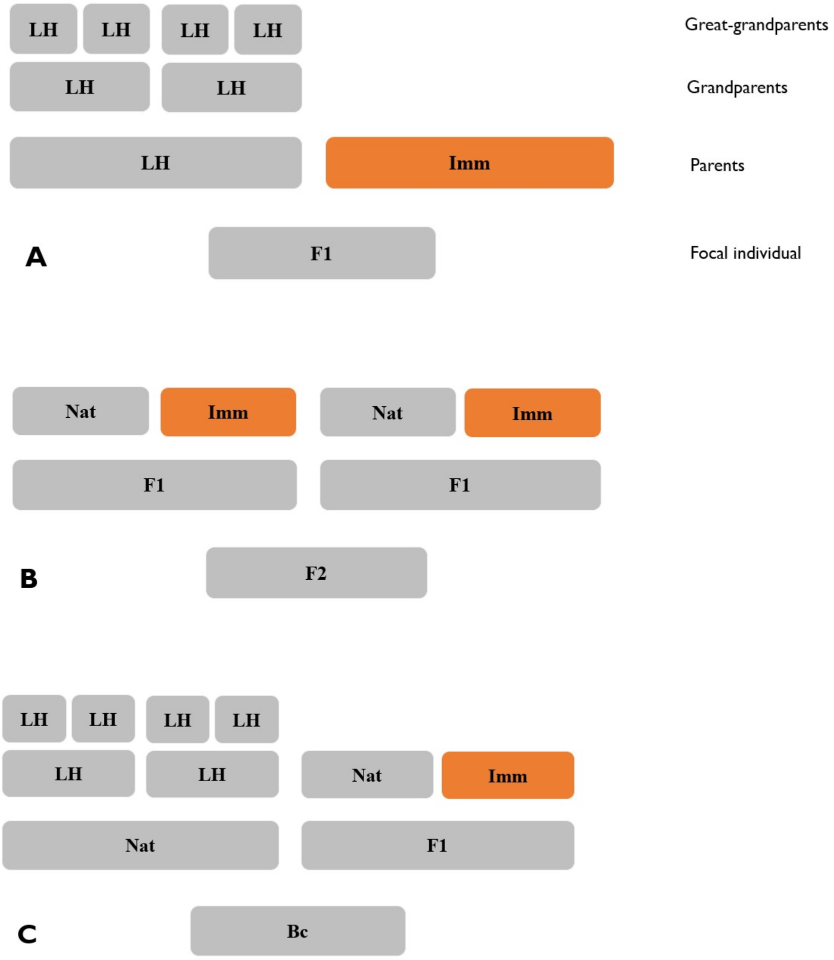


Table S2.1: Numbers of individuals with different types of parents (Parent status) that were pooled into the ‘other’ group for analyses of lifetime reproductive success (LRS), adult annual survival and reproductive success (Adult annual fitness components), juvenile survival and zygotes (egg) survival. Parent status gives the groups of a focal individual’s two parents, which can be immigrant (Imm), native (Nat), F1, F2 or backcross (Bc). Hence, for example, ‘Bc-F1’ denotes offspring of matings between Backcross and F1 individuals. For LRS, adult annual fitness components and juvenile survival, sample sizes denote numbers of individuals. For zygote survival, sample sizes denote numbers of clutches.

Parent status	**LRS**	**Adult annual fitness components**	**Juvenile survival**	**Zygote survival**
Bc-F1	40	48	211	85
Bc-Imm	7	8	67	27
F2-Bc	9	9	59	36
F2-F1	2	2	13	9
F2-Nat	20	20	79	50
Imm-F2	2	2	2	4
F2-F2	0	0	4	5
Imm-F1	0	0	0	12

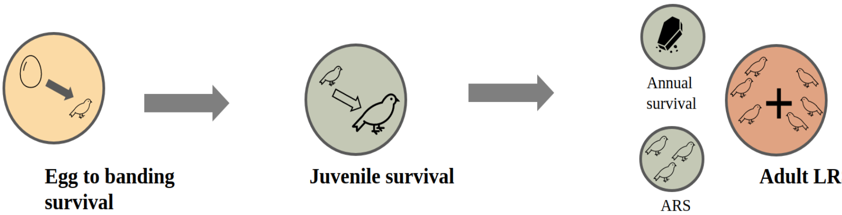
Supporting Information S3: Fitness measures and comparison to Marr et al. 2002

Measuring fitness components

Figure S3.1 defines key fitness components, and summarizes which years of data were used for each component. Fitness should ideally be measured from zygote-to-zygote, to avoid spanning generations, when comparing the fitness of subsequent generations in a line-cross framework. Our measures of zygote (i.e. egg) survival, juvenile survival and adult LRS are close to zygote-to-zygote overall fitness when combined, but slightly exceed this, as the overall measure runs from egg to banding at approximately six days old in the next generation. This measure was chosen because total eggs produced can be a misleading measure of fitness, since song sparrows can relay multiple times when consecutive clutches fail at early stages. Our estimates of between-group differences in ARS, LRS and overall fitness are consequently likely to be slightly conservative.

Due to our approach of modeling fitness components separately, distributions are not zero inflated and are well described by Poisson (for adult LRS and ARS) and Binomial (for zygote, juvenile and adult survival) distributions encompassing overdispersion, without requiring models specifically designed for zero-inflated data.

Figure S3.1: Definitions of focal fitness components measured, with the cohorts (for egg, zygote and juvenile survival and adult lifetime reproductive success) or years (adult annual reproductive success and survival) included in each analysis and notes on unavailable group and sex data. Lifetime reproductive success (LRS) is composed of adult annual survival and annual reproductive success (ARS). (*) Juvenile survival and adult annual survival were included for the last cohort and year (2018) surviving up to 2019.



Trait	**Egg to banding survival**	**Juvenile survival**	**Adult LRS**
Cohorts included	1983 - 2019	1993 – 2018 *	Adult LRS 1981 – 2016
Years included			Annual traits 1981 – 2018 *
Time span of trait	Egg laid – banding at day 6	Banding at day 6 – census in the following April (~age 1)	Survival as an adult to the next April census **(Annual survival)** Number of banded offspring produced each year **(ARS)** Results in total number of banded offspring produced **(adult LRS)**
Groups and sex	Immigrants missing No sex info	Immigrants missing	Sex included All groups included

Differences from Marr et al., 2002

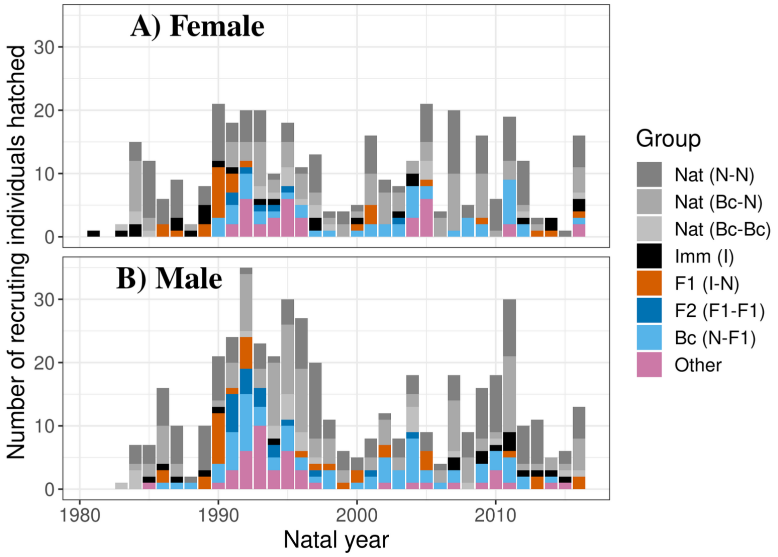
Marr et al. (2002) also aimed to estimate variation in fitness among immigrants and their descendants using the Mandarte song sparrow system. Our current analyses supersede this previous work in several key ways. Most obviously, we use an additional ~20 years of data, greatly reducing the challenges of small sample sizes that previously precluded strong statistical inference on key effects (Marr et al. 2002). Our hypothesis tests using planned comparisons among groups of individuals, and associated uncertainty on latent and phenotypic scale, are also greatly facilitated by the shift to Bayesian rather than frequentist analyses. We also treated survival traits as binomial variables, whereby Marr et al. (2002) used survival analyses (discrete-time proportional- hazards model). Further, our focal fitness components are defined within rather than across generations as far as feasible. In contrast, Marr et al. (2002) measured breeding success of focal adults as numbers of offspring that survived to independence from parental care (i.e. ca 24 days post hatch). This measure partially spans generations, and assigns chick survival to parents. Yet, our current analyses show that there are notable direct early life effects of an individual’s own heritage, implying that group effects could possibly be somewhat confounded, for example by including F2 effects in the estimation of F1 effects.

Our new analyses have strengthened some of the intriguing initial indications highlighted by Marr et al. (2002), including high F1 fitness and low F2 fitness. Yet, the enhanced data and different analytical decisions have altered some specific conclusions, for example that Marr et al. (2002) reported especially low F2 survival in males, whereas our analyses show low F2 survival in adult females.

Supporting Information S4: Data selection and checks for biases

We undertook additional analyses to check for possible biases and artifacts. First, we inspected the degree to which focal groups are distributed across study years (fig S4.1). Occurrences of F1 and F2 individuals are somewhat clustered, as is inevitable since their appearance depends on previous arrival of successful immigrants. Nevertheless, they are not confined to single years, and the broad temporal distribution of defined natives and backcross facilitates estimation of among-year variation. The small numbers of individuals in some groups in single years means that it is not feasible to estimate group by year interactions.

Figure S4.1: Numbers of (A) females and (B) males in each focal group that hatched in each study year. Visualized data are restricted to individuals that survived to recruit (age one year) and hence contribute to the dataset on adult fitness components, thereby representing the sparsest analysed dataset.



Second, we repeated all analyses after restricting to data from 1993 onward, representing the period when pedigree information was fully genetically verified, thereby eliminating any error in estimates of group membership and male ARS and LRS stemming from previous undetected extra-pair reproduction. Results and conclusions were qualitatively unchanged (estimates are not shown, but code to restrict data is provided).

Third, we also repeated analyses after excluding the lineage of one immigrant that previous microsatellite analyses suggested was distantly related to the native population at arrival (broadly comparable to an offspring of third-degree relatives, Dickel et al., 2021). This resulted in exclusion of a total of 31 individuals (the one immigrant and its 30 descendants), but conclusions remained unchanged.

Fourth, we considered whether the exclusion of breeding attempts where clutch sizes were not observed from analyses of zygote survival could have biased our core results regarding differences among focal groups. Such biases could arise if excluded attempts were predominantly from particular groups, meaning that estimates of zygote survival could be upwardly biased for these groups compared to others. However, there was no evidence of substantial variation in observed failure among groups (Table S4.1).

Table S4.1: Total numbers of unobserved and observed clutch sizes for each focal group across all study years, and the percentage of all clutch sizes in each group that were unobserved (columns) for groups of individuals as defined in Supporting Information S2: natives divided into three sub-groups comprising individuals with eight (N-N), seven (Bc-N) or six (Bc-Bc) locally hatched great-grandparents respectively (where N and Bc denote native and backcross grandparents), immigrants (Imm), F1s, F2s, backcrosses (Bc) and individuals which do not fall into the focal groups (other).

Group	**Number of clutch sizes unobserved**	**Number of clutch sizes observed**	**% unobserved**
Nat (N-N)	49	894	5.2
Nat (Bc-N)	15	459	3.2
Nat (Bc-Bc)	3	107	2.7
F1 (I-N)	1	119	0.8
F2 (F1-F1)	2	64	3.0
Bc (N-F1)	7	243	2.8
Other	9	260	3.4

Fifth, to examine whether there could be group by sex interactions on zygote survival, given that sexes of zygotes that did not survive to banding are unknown, we calculated the sex ratios of banded chicks in each group. Assuming a 1:1 sex ratio at conception, biased sex ratio within any group at banding could imply sex-specific survival to that stage. However there was no evidence of any such effects: sex ratios of observed chicks within each group did not differ substantially from 1:1.

Supporting Information S5: Models, data summaries and model coefficients

All planned comparisons were computed from posterior distributions of estimated group effects, not by combining groups within the primary GLMMs to directly estimate mean effects, thereby ensuring that estimates are not biased by highly unbalanced group sample sizes. For example, we did not directly estimate the $\mu(I,NN)$ effect by pooling the immigrants and native-native into a single parental group within the original model; since natives substantially outnumber immigrants resulting estimates would primarily reflect native effects.

Totals of 1,006,000 posterior samples were collected for all models with burn in 6000 and thinning interval 500, resulting in approximately 3000 effective posterior samples. We ensured that autocorrelations were lower than 0.05, and multiple model re-runs yielded quantitatively similar answers, implying similar convergence of posterior distributions.

To facilitate future comparative analyses, we present the raw means and standard deviations of all fitness components in Table S5.1. We then present posterior distributions of model-estimated parameters as their means and 95% credible intervals on the latent scale (Table S5.2) and phenotypic scale (Table S5.4) for the fixed (group) effects, and on the latent scale for the random effects (Table S5.3). We additionally present the estimated age effects of age for adult annual reproductive success (ARS) and survival probability (Table S5.5).

Table S5.1: Raw data means and standard deviations (SD) of fitness components for females (F) and males (M) of each focal group. Columns indicate groups of individuals, sex, and the five quantified fitness components: Lifetime reproductive success (LRS), adult annual reproductive success (ARS) and survival, and juvenile and zygote survival. Rows contain raw means for each fitness components for groups of individuals as defined in Supporting Information S2: natives divided into three sub-groups comprising individuals with eight (N-N), seven (Bc-N) or six (Bc-Bc) locally hatched great-grandparents respectively (where N and Bc denote native and backcross grandparents), immigrants (Imm), F1s, F2s, backcrosses (Bc) and individuals which do not fall into the focal groups (other). Each fitness component's row is split into females (F) and males (M) except for zygote survival, where sexes are combined. Dashes indicate absence of data for immigrants at pre-dispersal stages (i.e. juvenile and zygote survival).

Group	**Sex**	**LRS**	**ARS**	**Annual survival**	**Juvenile survival**	**Zygote survival**
Nat (N-N)	**F**	10.0±9.1	4.6±2.7	0.54±0.50	0.17±0.38	0.53±0.42
	M	7.9±9.1	3.6±3.7	0.56±0.50	0.20±0.40	
Nat (Bc-	**F**	10.6±9.3	4.7±2.5	0.56±0.50	0.18±0.39	

N)	**M**	7.2±8.4	2.9±3.1	0.59+0.49	0.24±0.43	0.62±0.40
Nat (Bc-Bc)	**F**	10.3±6.8	4.2±2.5	0.59±0.50	0.16±0.37	0.66±0.41
	M	8.5±9.3	3.1±3.7	0.63±0.49	0.19±0.39	
Imm (I)	**F**	10.4±7.7	5.1±3.0	0.51±0.51	-	-
	M	6.9±9.2	3.2±3.5	0.54±0.51	-	
F1 (I-N)	**F**	14.3±12.1	5.7±3.0	0.60±0.49	0.15±0.36	0.73±0.37
	M	17.3±15.2	4.9±3.9	0.71±0.45	0.27±0.44	
F2 (F1-F1)	**F**	6.1±2.1	5.4±1.2	0.12±0.35	0.13±0.35	0.51±0.43
	M	6.0±7.2	2.2±2.9	0.63±0.49	0.31±0.47	
Bc (N-F1)	**F**	9.9±10.2	4.9±2.7	0.51±0.50	0.17±0.38	0.71±0.36
	M	8.9±9.9	3.5±3.4	0.60±0.49	0.25±0.43	
Other	**F**	10.9±9.7	5.1±2.0	0.53±0.50	0.12±0.32	0.62±0.40
	M	8.0±10.0	3.4±3.7	0.58±0.50	0.18±0.3	

Table S5.2: Estimated means [and 95% credible intervals] for group effects on latent scale for females (F) and males (M) of groups of individuals (rows) and fitness components (columns). Table structure is as in Table S5.1.

Group	**Se**	**LRS**	**ARS**	**Annual**	**Juvenile**	**Zygote**

	x	[95%CI]	[95%CI]	survival [95%CI]	survival [95%CI]	survival [95% CI]
Nat (N-N)	F	1.9 [1.7; 2.2]	1.6 [1.5; 1.8]	0.32 [-0.2; 0.80]	-1.94 [-2.61; -1.30]	0.67 [0.22; 1.15]
	M	1.5 [1.3; 1.7]	1.3 [1.1; 1.5]	0.42 [0.04; 0.95]	-1.69 [-2.35; -1.05]	
Nat (Bc-N)	F	2.0 [1.5; 2.4]	1.7 [1.5; 2.0]	0.33 [-0.32; 1.00]	-2.00 [-2.84; -1.17]	1.20 [0.58; 1.84]
	M	1.4 [1.0; 1.8]	1.2 [0.9; 1.4]	0.53 [-0.12; 1.15]	-1.46 [-2.27; -0.68]	
Nat (Bc-Bc)	F	2.3 [1.7; 2.9]	1.8 [1.4; 2.1]	0.53 [-0.42; 1.51]	-2.11 [-3.18; -1.07]	1.72 [0.80 ; 2.66]
	M	1.6 [1.0; 2.2]	1.2 [0.9; 1.5]	0.76 [-0.11; 1.64]	-1.81 [-2.80; -0.83]	
Imm (I)	F	2.1 [1.4; 2.7]	1.8 [1.4; 2.2]	0.12 [-0.84; 1.09]	-	-
	M	1.0 [0.2; 1.8]	1.1 [0.6; 1.5]	0.47 [-0.60; 1.53]	-	
F1 (I-N)	F	2.2 [1.6; 2.7]	1.7 [1.4; 2.1]	0.41 [-0.44; 1.31]	-2.30 [-3.43; -1.19]	2.02 [1.12; 2.90]
	M	2.3 [1.8;	1.7 [1.4;	1.22 [0.43;	-1.35 [-2.37; -	

		2.8]	2.0]	1.97]	0.35]	
F2 (F1-F1)	F	1.7 [0.7; 2.6]	2.2 [1.6; 2.8]	-3.29 [-6.86; -0.88]	-2.94 [-4.59; -1.32]	0.28 [-0.88; 1.49]
	M	1.0 [0.3; 1.7]	0.9 [0.5; 1.3]	0.57 [-0.37; 1.52]	-1.22 [-2.65; 0.10]	
Bc (N-F1)	F	1.9 [1.4; 2.3]	1.8 [1.5; 2.0]	0.12 [-0.66; 0.90]	-2.15 [-3.07; -1.28]	1.73 [0.98; 2.49]
	M	1.5 [1.1; 2.0]	1.3 [1.0; 1.5]	0.46 [-0.20; 1.13]	-1.42 [-2.26; -0.60]	
Other	F	2.2 [1.6, 2.7]	1.9 [1.6; 2.2]	0.18 [-0.67; 0.99]	-2.55 [-3.48; -1.68]	0.84 [0.11; 1.59]
	M	1.4 [1.0; 1.9]	1.3 [1.0; 1.6]	0.34 [-0.38; 1.06]	-2.05 [-2.91; -1.19]	

Table S5.3: Random effect estimates on the latent scale with 95% credible intervals (CI) [in square brackets] for models of lifetime reproductive success (LRS), annual reproductive success (ARS) and survival and juvenile and zygote survival. Rows indicate the different random effects where a dashes (-) indicate if the random effect was not included in a particular model. Name in parentheses indicates the variable name used in the model code. Pair ID was included in all models to account for possible non-independence of observations within cohorts, years and parent pairings (i.e. parents of each focal individual). Annual adult traits contained repeated observations per individual and therefore contained random individual identity (ID) and year effects. Juvenile survival was modeled at the individual level, with random nest ID effects. Zygote survival was directly modeled at the clutch level, hence random nest ID effects were not included.

Group	**LRS [95%CI]**	**ARS [95%CI]**	**Annual survival [95%CI]**	**Juvenile survival [95%CI]**	**Zygote survival [95%CI]**
Pair ID	0.02 [0.00; 0.11]	0.01 [0.00; 0.04]	0.04 [0.00; 0.16]	0.41 [0.07; 0.82]	0.83 [0.34; 1.38]
Natal year	0.15 [0.06; 0.31]	-	-	1.87 [0.92; 3.64]	1.28 [0.66; 2.22]
Year	-	0.18 [0.10; 0.31]	0.92 [0.48; 1.63]	-	-
Nest ID	-	-	-	0.59 [0.07; 1.16]	-
ID	-	0.12 [0.08; 0.17]	0.07 [0.00; 0.34]	-	-

Table S5.4: Estimated means and 95% credible intervals (CI) [in square brackets] for group effects on phenotypic scale for females (F) and males (M) for all fitness components. Table structure and elements are the same as in Table S5.1.

Group	**Sex**	**LRS [95%CI]**	**ARS [95%CI]**	**Annual Survival [95%CI]**	**Juvenile survival [95%CI]**	**Zygote survival [95% CI]**
Nat (N-N)	F	6.9 [5.4; 8.7]	5.2 [4.3; 6.2]	0.58 [0.45; 0.69]	0.13 [0.07; 0.21]	0.66 [0.56; 0.76]
	M	4.6 [3.6; 5.7]	3.5 [2.9; 4.3]	0.60 [0.49; 0.72]	0.16 [0.09; 0.26]	
Nat (Bc-N)	F	7.4 [4.6; 11.1]	5.6 [4.4; 7.0]	0.58 [0.42; 0.72]	0.13 [0.06; 0.24]	0.76 [0.64; 0.86]
	M	4.1 [2.7; 5.9]	3.2 [2.5; 4.0]	0.63 [0.47; 0.76]	0.20 [0.09; 0.34]	
Nat (Bc-Bc)	F	10.3 [5.2; 18.3]	5.9 [4.1; 8.1]	0.62 [0.40; 0.82]	0.12 [0.04; 0.26]	0.84 [0.80; 0.93]
	M	5.3 [2.8; 9.1]	3.4 [2.4; 4.6]	0.67 [0.47; 0.84]	0.15 [0.06; 0.30]	
Imm	F	8.4 [4.1; 15.4]	6.1 [4.1; 9.0]	0.53 [0.30; 0.75]	-	-

(I)	M	2.9 [1.3; 5.8]	3.0 [1.9; 4.5]	0.61 [0.35; 0.82]	-	
F1 (I-N)	F	9.1 [4.9; 15.1]	5.7 [4.1; 7.7]	0.60 [0.39; 0.79]	0.10 [0.03; 0.23]	0.88 [0.75; 0.95]
	M	10.3 [6.0; 16.6]	5.6 [4.2; 7.3]	0.77 [0.61; 0.88]	0.22 [0.09; 0.41]	
F2 (F1-F1)	F	6.1 [2.1; 13.8]	9.5 [5.2; 15.7]	0.07 [0.00; 0.29]	0.07 [0.01; 0.21]	0.57 [0.29; 0.82]
	M	3.0 [1.4; 5.5]	2.5 [1.7; 3.5]	0.63 [0.41; 0.82]	0.25 [0.07; 0.53]	
Bc (N-F1)	F	6.6 [3.9; 10.1]	5.8 [4.4; 7.6]	0.53 [0.34; 0.71]	0.11[0.04; 0.22]	0.84 [0.73; 0.92]
	M	4.8 [3.0; 7.2]	3.6 [2.8; 4.7]	0.61 [0.45; 0.76]	0.20 [0.09; 0.35]	
Other	F	8.9 [5.2; 14.4]	6.8 [5.1; 9.0]	0.54 [0.34; 0.73]	0.08 [0.03; 0.16]	0.69[0.53;0.83]
	M	4.3 [2.6; 6.9]	3.7 [2.8; 4.7]	0.58 [0.41; 0.74]	0.12 [0.05; 0.23]	

Table S5.5: Age effect estimates for models of annual survival and annual reproductive success on latent and phenotypic scale (columns) for different age groups (rows). The intercept indicates mean annual survival and reproductive success for a native (N-N) female with the 95%CI shown in square brackets. “Old” and “Young” then represent a native female of the respective age groups. Age effects were estimated across all groups of individuals, i.e. they can be equally added to all groups and sexes.

Age category	**Annual survival**		**Annual reproductive success (ARS)**	
Scale	**Latent**	**Phenotypic**	**Latent**	**Phenotypic**
Intercept	0.31 [-0.16; 0.78]	0.58 [0.46; 0.69]	1.6 [1.5; 1.8]	5.2 [4.3; 6.2]
Old	-0.56 [-1.40; 0.17]	0.37 [0.20; 0.54]	1.2 [1.0; 1.5]	3.5 [2.7; 4.4]
Young	0.33 [-0.14; 0.79]	0.58 [0.46; 0.69]	1.1 [0.9; 1.3]	3.0 [2.5; 3.6]

Supporting Information S6: Alternative overall fitness approximations

We calculated two additional approximations of overall fitness using alternative estimates of juvenile survival. First, to eliminate any among-group variation that could have resulted from differences in dispersal probabilities, we fitted an additional model estimating juvenile survival as a sex-specific constant across groups. We then multiplied resulting estimates of sex-specific juvenile survival probabilities with lifetime reproductive success and zygote survival equivalent to the analyses presented in the main manuscript. We compared both total fitness approximations (fig. S6.1, where the red lines represent the results with constant juvenile survival). Effects remained qualitatively similar, but became quantitatively slightly more conservative as expected, because group differences were reduced through constant juvenile survival.

Second, to draw further conclusions regarding unobservable immigrant effects on pre-dispersal states, we extracted the previously estimated additive genetic effect of immigrants on juvenile survival in the focal song sparrow population (from Reid et al., 2021). We then approximated a hypothetical immigrant value for juvenile survival by subtracting the negative effect of immigrant ancestry from the natives’ (N-N) posterior distribution, and used this value as the immigrants’ juvenile survival (fig. S6.2A, B). We then repeated the planned comparisons including the approximated value for juvenile survival (fig S6.2C,D,E). These analyses suggested that, given such low (hypothetical) juvenile survival in immigrants, there could be some local heterosis in juvenile survival in males.

To obtain an overall fitness approximation with the additive value for juvenile survival of immigrants, we then multiplied the obtained juvenile survival value with the observed LRS of immigrants, and zygote survival of the native group as a conservative baseline assumption (fig S6.3A,B). We then repeated the planned comparisons including the projected immigrant effects, showing that with the immigrants' additive genetic values, there could be strong heterosis in males and epistatic breakdown in both sexes.

Fig. S6.1: Posterior distributions of (A, B) overall fitness approximated as the product of zygote and juvenile survival probability and adult LRS for (A) females and (B) males; and planned comparisons between selected groups for (C) females, (D) males, and (E) the between-sex difference. Filled posterior distributions show the overall fitness approximation with group-specific juvenile survival presented in the main manuscript. Red lines show the posterior distributions with constant juvenile survival. A and B show posterior distributions of the overall fitness approximation for natives (light grey) divided into three sub-groups comprising individuals with eight (N-N), seven (Bc-N) or six (Bc-Bc) locally hatched great-grandparents respectively (where N and Bc denote native and backcross grandparents), F1s (orange), F2s (dark blue), and backcrosses (light blue). Immigrants are not present during pre-dispersal life stages (i.e. as eggs and juveniles) in the local population. Hence, there is no posterior distribution for immigrants on A and B. C and D show the posterior distributions of differences between F1s and natives($\Delta_{F1\text{-}NN}$), and between F2s and μ(F1,NN) ($\Delta_{F2\text{-}\mu(F1,NN)}$), with no immigrant component. In C-E, light blue versus grey highlight the proportions of the posterior distributions above versus below zero. On all panels, black points denote posterior medians, and thick and thin horizontal bars denote the 50% and 95% Cis.

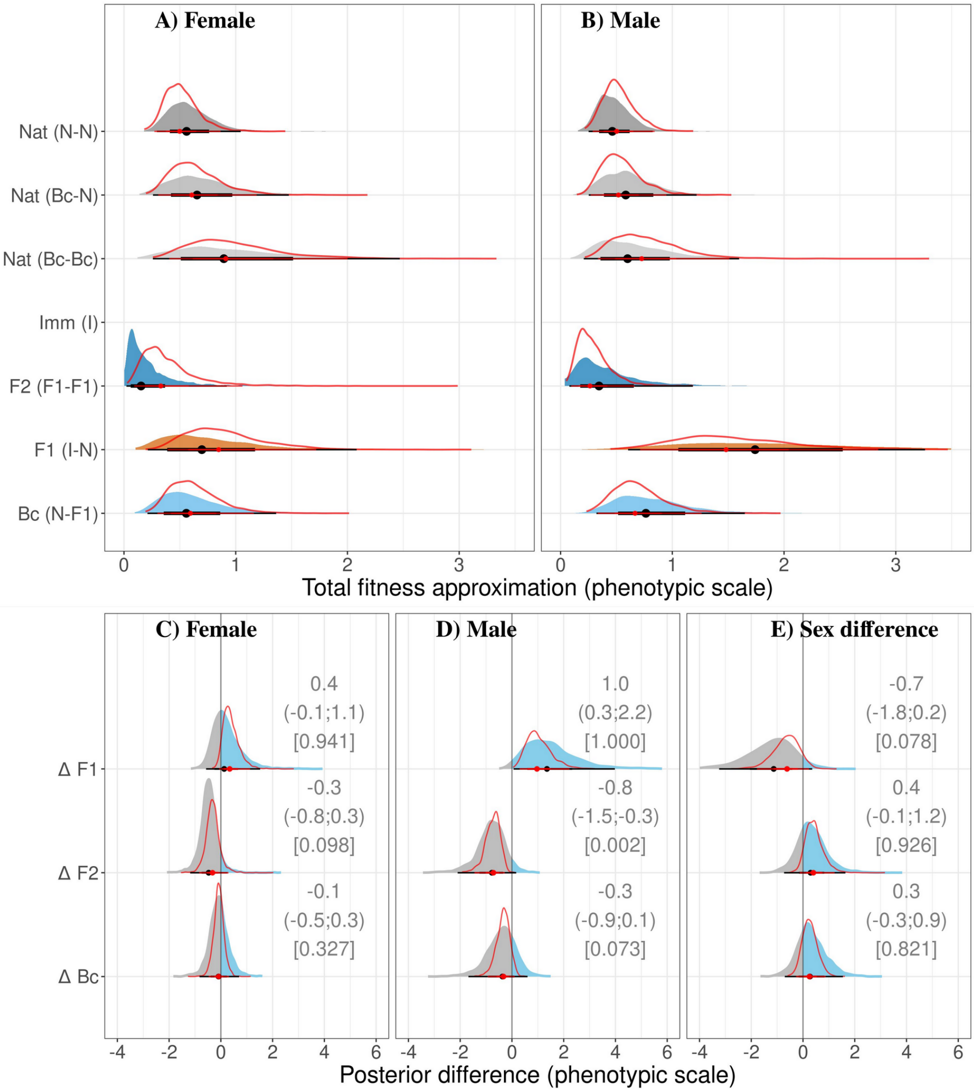

A) Female
B) Male
Nat (N-N)
Nat (Bc-N)
Nat (Bc-Bc)
Imm (I)
F2 (F1-F1)
F1 (I-N)
Bc (N-F1)
0
1
2
3
Total fitness approximation (phenotypic scale)
C) Female
D) Male
E) Sex difference
Δ F1
Δ F2
Δ Bc
0.4
(-0.1;1.1)
[0.941]
-0.3
(-0.8;0.3)
[0.098]
-0.1
(-0.5;0.3)
[0.327]
1.0
(0.3;2.2)
[1.000]
-0.8
(-1.5;-0.3)
[0.002]
-0.3
(-0.9;0.1)
[0.073]
-0.7
(-1.8;0.2)
[0.078]
0.4
(-0.1;1.2)
[0.926]
0.3
(-0.3;0.9)
[0.821]
-4
-2
0
2
4
6
Posterior difference (phenotypic scale)

Figure S6.2: Posterior distributions of (A, B) juvenile survival probability on the phenotypic scale for (A) females and (B) males; and planned comparisons between selected groups for (C) females, (D) males, and (E) the between-sex difference with an approximated juvenile survival for immigrants (red posterior distribution) based on Reid et al. (2021). All elements are as in fig. 5 in the main manuscript except for the added juvenile survival approximation (panel A and B), and the subsequently changed planned comparisons, which are here highlighted in red (Panel C-E).

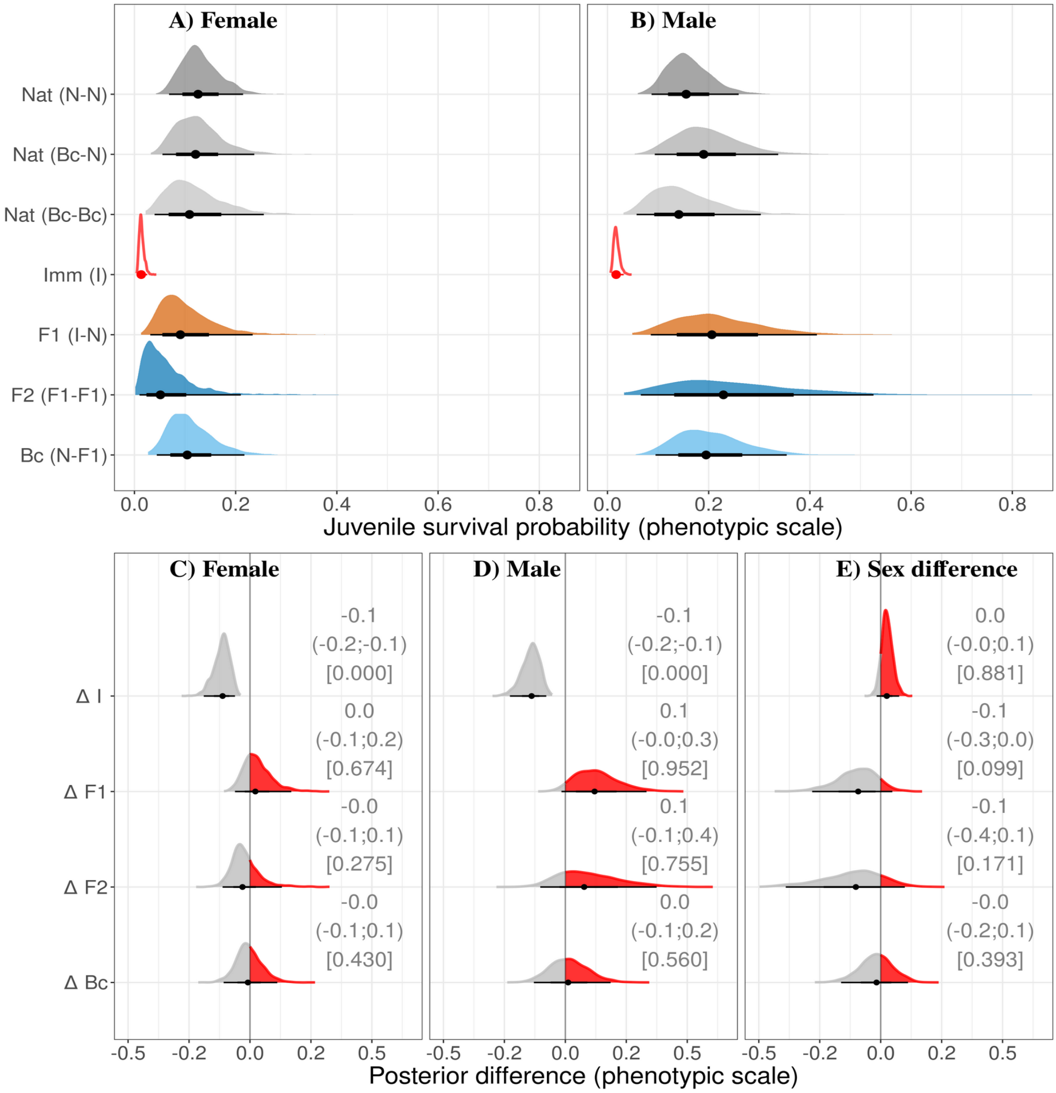
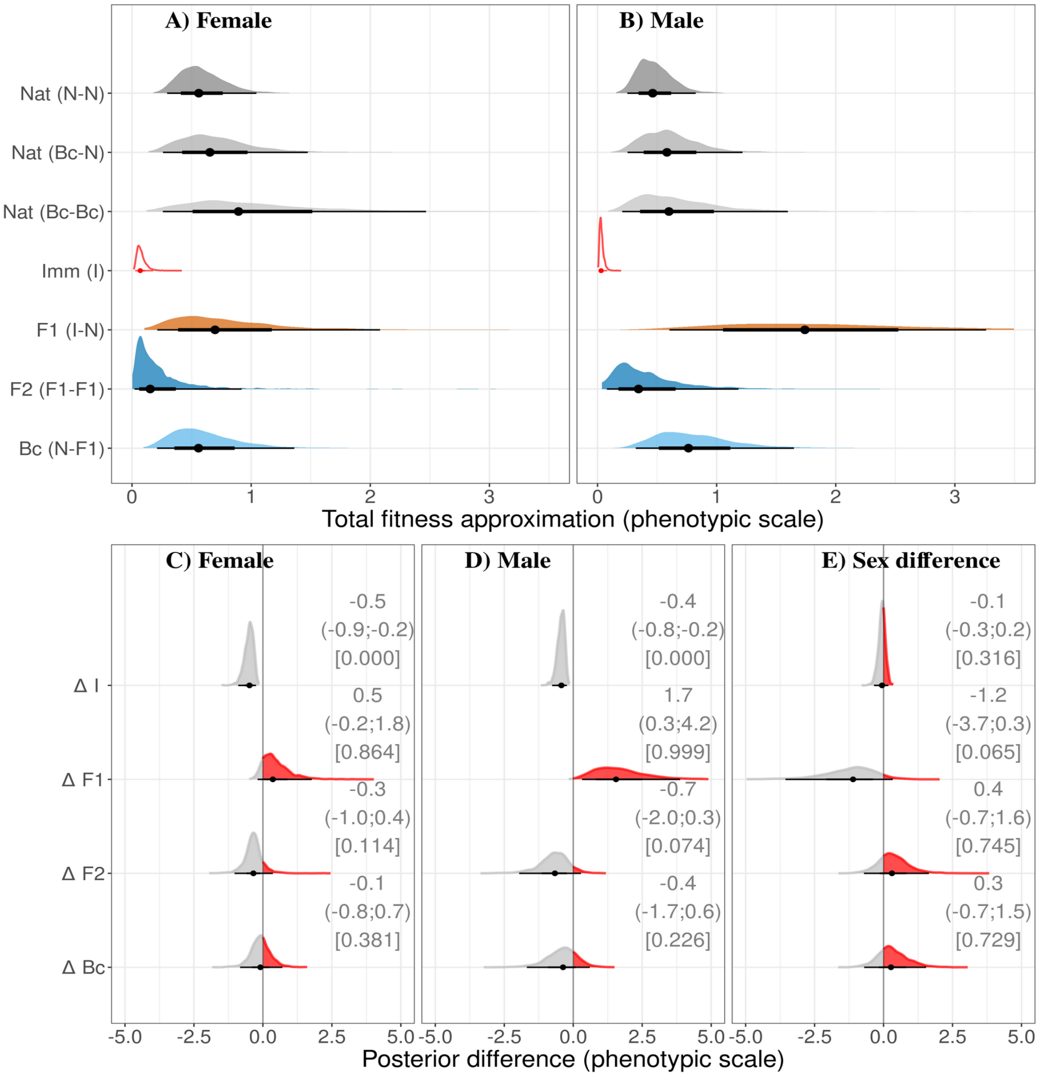


Figure S6.3: Posterior distributions of (A, B) overall fitness approximated as the product of zygote and juvenile survival probability and adult LRS for (A) females and (B) males; and planned comparisons between selected groups for (C) females, (D) males, and (E) the between-sex difference. The approximation of juvenile survival based on Reid et al., 2021 has been used for all planned comparisons, which are here highlighted in red. All other elements of the figure are as in fig. 7 of the main manuscript.



Supporting Information S7: Hypothetical immigrant values

To assess the robustness of our inferences and conclusions on heterosis and epistatic breakdown given that immigrants' values for some fitness components are not locally observable, or could be confounded by developmental environmental effects, we calculated hypothetical values for immigrants that would be required to generate the values observed for other groups in the absence of epistatic and/or dominance effects.

First, we derived the immigrant values that would be required to make observed values for F1s and F2s feasible given exclusively additive genetic effects, and given additive and dominance effects with no epistasis (Table S7.1). Here, $F1 = F2 = \mu(I, N)$ by definition given purely additive effects, which can be rearranged to give $I = 2F1-N$ and $I = 2F2-N$ respectively (Table 7.1). Then, $F2 = \mu(F1, \mu(N,I)$ given additive and dominance effects, which can be rearranged to give $I = 4F2-2F1-N$ (Table S7.1). We then used these equations to compute the full posterior distributions of hypothetical immigrant values, given the estimated posterior distributions of the necessary groups (natives, F1s, F2s). We first carried out all calculations on the originally modeled, latent scale (Table S7.2), and then back-transformed them onto the phenotypic scale (Table S7.3).

Table S7.1: Equations to infer immigrant values to produce observed F1 and F2 values given (A,B) additive and (C) additive and dominance values, including the initial form of the equation from line cross theory, and re-arranging to isolate the immigrant value.

Comparison	**Equation**
(A) Additive genetic effects assumption for F1s	$F1 = \mu(I, N)$ $F1 = (I + N)/ 2$ $\mathbf{I = 2F1 - N}$
(B) Additive genetic effects assumption for F2s	$F2 = \mu(I, N)$ $F2 = (I + N)/2$ $\mathbf{I = 2F2 - N}$
(C) Additive and dominance genetic effects assumption	$F2 = \mu(F1, \mu(N, I)$ $F2 = (F1 + (N+ I)/2) / 2$ $\mathbf{I = 4F2 - 2F1 - N}$

Results are presented in Table S7.2. We found that most hypothetical values for fitness components of immigrants that would be required to generate observed results without dominance and epistasis exceed biologically plausible ranges, with survival probabilities close to 0 and 1 and reproductive values exceeding feasible and observed mean values (Table S7.2, S7.3). Specifically explaining our results with only additive genetic effects would require immigrants to have zygote survival probability of 0.95, male LRS of

25.5 offspring, and male annual survival probability of 0.86 (Table S7.3A). Furthermore, entirely different immigrant values would be required to produce observed F2 values given only additive genetic effects (Table S7.2B, S7.3B). Then, explaining our results exclusively with additive and dominance genetic effects would require immigrants to have zygote survival probability of 0.12 and female juvenile survival probability of 0.09, female annual reproductive success of 80.95 and female annual survival probability of 0.00 to produce the observed F1 and F2 values given only additive and dominance genetic effects (Table S7.2C, S7.3C).

Table S7.2: Hypothetical immigrant values on modeled, latent scales based on equations A-C (Table S7.1, columns), presented as posterior means and 95% credible intervals (CI) in square brackets for each fitness component, i.e. zygote and juvenile survival, lifetime reproductive success (LRS), annual reproductive success (ARS), and annual survival. Rows are split by ‘Sex’ for females (F) and males (M). Sexes are combined for the zygote stage, because sex information is not available.

Fitness component	**Sex**	**(A) Expected value under additive model F1 = μ(I, N)**	**(B) Expected value under additive model F2 = μ(I, N)**	**(C) Expected value under dominance model F2 = μ(F1, μ(N, I)**

Zygote survival	-	3.37 [1.50; 5.16]	-0.11 [-2.52; 2.34]	-3.59 [-8.53; 1.44]
Juvenile survival	F	-2.66 [-5.01; -0.33]	-3.93 [-7.41; -0.67]	-5.20 [-12.05; 1.48]
	M	-1.02 [-3.19; 1.14]	-0.75 [-3.67; 2.00]	-0.49 [-6.41; 5.07]
LRS	F	2.4 [1.2; 3.5]	1.5 [-0.4; 3.3]	0.5 [-3.4; 4.3]
	M	3.1 [2.0; 4.1]	0.6 [-0.8; 1.9]	-2.0 [-4.7; 1.0]
ARS	F	1.8 [1.2; 2.5]	2.8 [1.6; 3.9]	3.8 [1.5; 6.0]
	M	2.2 [1.6; 2.7]	0.6 [-0.2; 1.3]	-1.1 [-2.6; 0.4]
Annual survival	F	0.49 [-1.25; 2.29]	-6.90 [-13.80; -2.10]	- 14.30 [-27.99; -4.72]
	M	2.02 [0.40; 3.54]	0.72 [-1.21; 2.70]	0.58 [4.54; 3.38]

Table S7.3: Hypothetical immigrant values on back-transformed, phenotypic scales based on equations A-C (columns), presented as posterior means and 95% credible intervals (CI) in square brackets for each fitness component (rows), which are split by ‘Sex’ for females (F) and males (M) except for zygote survival, because sex information is not available. All table elements are as in Table S7.2.

Fitness component	**Sex**	**(A) Expected value under additive model F1 = μ(I, N)**	**(B) Expected value under additive model F2 = μ(I, N)**	**(C) Expected value under dominance model F2 = μ(F1, μ(N, I)**
Zygote	-	0.95 [0.82; 0.99]	0.48 [0.07; 0.91]	0.12 [0.00; 0.81]
Juvenile survival	F	0.10 [0.01; 0.42]	0.05 [0.00; 0.34]	0.09 [0.00; 0.81]
	M	0.31 [0.04; 0.76]	0.37 [0.02; 0.88]	0.44 [0.00; 0.99]
LRS	F	13.3 [3.3; 34.4]	7.0 [0.6; 28.2]	10.9 [0.0; 76.5]
	M	25.5 [7.6; 61.2]	2.3 [0.4; 6.6]	0.4 [0.0; 2.6]
ARS	F	6.5 [3.3; 12.0]	19.3 [5.1; 50.0]	80.95 [4.4; 405.49]
	M	9.01 [5.0; 15.1]	1.9 [0.8; 3.6]	0.5 [0.1; 1.4]
Annual survival	F	0.60 [0.22; 0.91]	0.01 [0.00; 0.11]	0.00 [0.00; 0.01]
	M	0.86 [0.60; 0.97]	0.65 [0.23; 0.93]	0.41 [0.01; 0.97]

Supporting Information S8: Results accounting for pedigree *f*

Strong inbreeding depression might explain the observed heterosis in the focal population. Further, fitness of subsequent generations after immigration can decrease, for example due to inbreeding among descendants of successful immigrants (Kardos et al., 2018). Despite these expectations, we deliberately did not explicitly model effects of individual coefficient of inbreeding (*f*) in our main analyses, because doing so would likely control for part of the group effects we aim to estimate, since mean *f* differs among the focal groups. Nevertheless, an interesting secondary question concerns the degree to which the estimated group effects can be explained by variation in the degree of local inbreeding. To examine this, we refitted the models presented in the main manuscript, with an additional sex-specific regression on pedigree *f* (i.e. sex by *f* interaction). For further detailed explanation of pedigree construction and calculation of *f*, see Nietlisbach et al., (2017).

Analyses showed that estimated group effects in fact remained qualitatively similar when regressions on *f* were included (fig. S8.1-S8.5, Table S8.1). The greatest changes were evident in the estimates for native males (particularly LRS, fig. S8.1, ARS, fig. 8.3, and juvenile survival, fig. 8.4, Table S8.1). Here, predicted values of fitness components were higher after accounting for *f*, effectively reflecting the inbreeding depression within each group.

Estimated slopes (Table S8.1) differ from previous estimates of inbreeding depression in the song sparrow system (Keller et al., 2008; Reid et al. 2011, Nietlisbach et al., 2017, Wolak et al., 2018), because part of the variation in inbreeding is captured by the groups. For some groups, there is no variation in f because we assume $f = 0$ for immigrants and F1s. Hence the estimated effects for these groups do not change whether or not a regression on f is modelled.

Reversal of inbreeding depression only explained a minor part of heterosis, and hence did not change the results qualitatively, consistent with the theory (Whitlock et al., 2000, Charlesworth, 2018). However, pedigree f does not capture the full range of variation in inbreeding. This is because f is calculated relative to the local population's pedigree baseline, assuming $f = 0$ for immigrants and F1s. Yet, F1 individuals could in fact be more outbred than assumed, if new immigrants are less related to pedigree founders than the founders are to each other (Dickel et al., 2021). Multi-population or genomic estimates of f might consequently explain more heterosis than the local pedigree f.

On Figures S8.1-S8.5 the red lines show posterior distributions of the group effects when a regression on individual f was included in the model. These posterior distributions represent the expected group effect given $f = 0$, and are shown in comparison to the estimates without a regression on f.

Table S8.1: Estimated slopes of regression on *f* for LRS, ARS, annual, juvenile and egg survival for males and females for all fitness components except egg survival, where there is no sex information available. All estimates are presented on the latent scales (i.e. log scale for LRS and ARS; logit scale for survival probabilities).

Fitness component	**Sex**	**Mean**	**Median**	**Mode**	**Lower 95 CI**	**Upper 95 CI**
LRS	Female	-0.15	-0.15	-0. 62	-3.19	2.90
LRS	Male	-3.59	-3.59	-4.00	-6.12	-1.03
ARS	Female	-0.13	-0.14	-0.26	-1.67	1.46
ARS	Male	-2.32	-2.31	-2.23	-3.65	-0.95
Annual survival	Female	1.38	1.45	1.47	-3.21	5.77
Annual survival	Male	-1.53	-1.56	-1.86	-4.92	2.02
Juvenile survival	Female	-8.34	-8.33	-8.83	-13.72	-3.34
Juvenile survival	Male	-10.49	-10.56	-10.59	-15.42	-5.69

Egg survival	Combined	-0.49	-0.48	-0.48	-3.65	2.69

Figure S8.1: Posterior distributions of (A,B) adult lifetime reproductive success (LRS, total number of chicks produced) for each focal group of individuals in (A) females and (B) males, and (C,D,E) planned comparisons between selected groups. Filled posterior distributions show LRS estimated by the model presented in the main manuscript, while the red lines show the posterior distributions from models that additionally include sex-specific regressions on coefficient of inbreeding (*f*). A and B show posterior distributions of adult LRS for natives (light grey) divided into three sub-groups comprising individuals with eight (N-N), seven (Bc-N) or six (Bc-Bc) locally hatched great-grandparents respectively (where N and Bc denote native and backcross grandparents), immigrants (dark grey), F1s (orange), F2s (dark blue), and backcrosses (light blue). C and D show posterior distributions of differences, whereby ΔI represents the difference between immigrants and natives (ΔI-NN), ΔF1 the difference between F1s and μ(I,NN), ΔF2 the difference between F2s and μ(F1,μ(I,NN) (ΔF2), and ΔBc between backcrosses and μ(F1, NN) (table 1, main text), for (C) females and (D) males. E shows the posterior distributions of the difference between females and males for each of the four comparisons. Light blue versus grey highlight the proportions of the posterior distributions above versus below zero. On all panels, black points denote posterior medians, and thick and thin horizontal bars denote the 50% and 95% CIs. Filled posterior distributions show adult lifetime reproductive success estimated by the model presented in the main manuscript, while the red lines show the posterior distributions of the identical model with sex-specific regression on *f*.

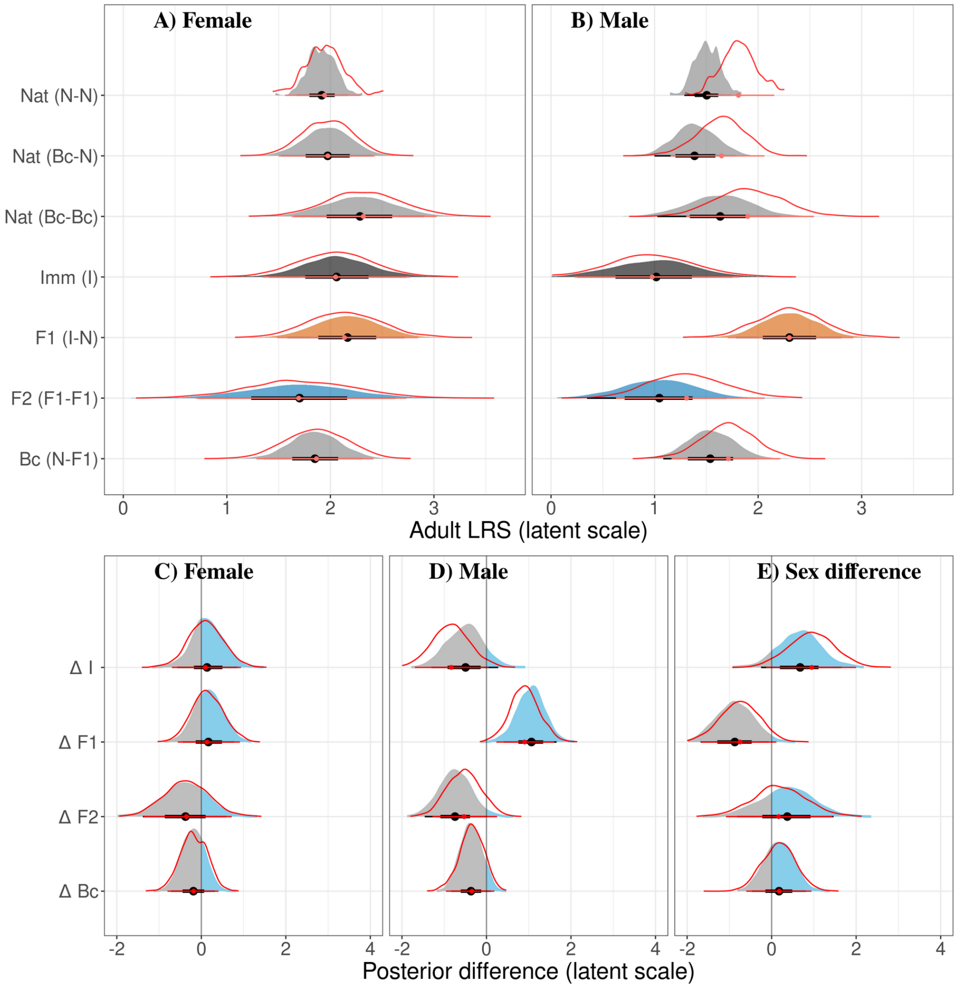


Figure S8.2: Posterior distributions of (A, B) annual survival of each focal group of individuals, and (C, D, E) planned comparisons between selected groups. Figure structure and attributes as in Figure S8.1. Filled posterior distributions show annual survival probability estimated by the model presented in the main manuscript, while the red lines show the posterior distributions of the identical model with sex-specific regressions on *f*.

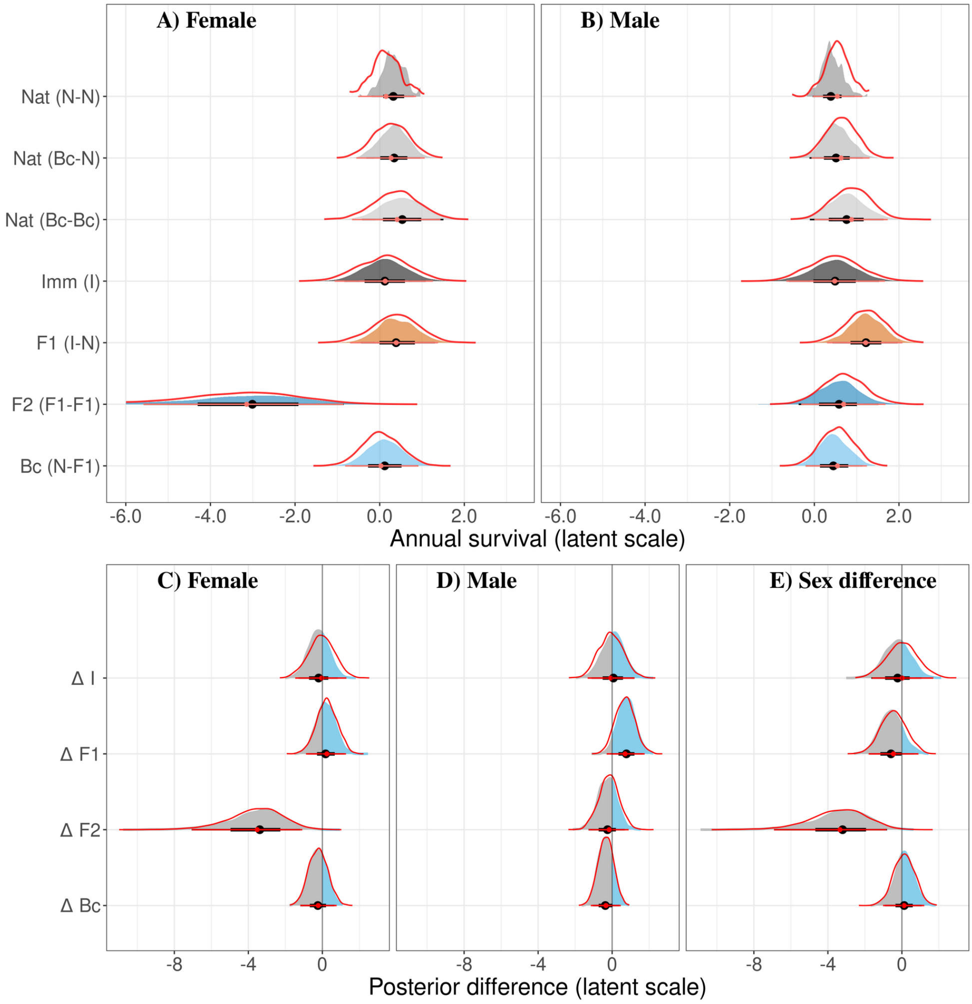


Figure S8.3: Posterior distributions of (A,B) annual reproductive success of each focal group of individuals and (C,D,E) planned comparisons between selected groups. Figure structure and attributes are as in Figure 2. Filled posterior distributions show annual reproductive success estimated by the model presented in the main manuscript, while the red lines show the posterior distributions of the identical model with sex-specific regressions on *f*.

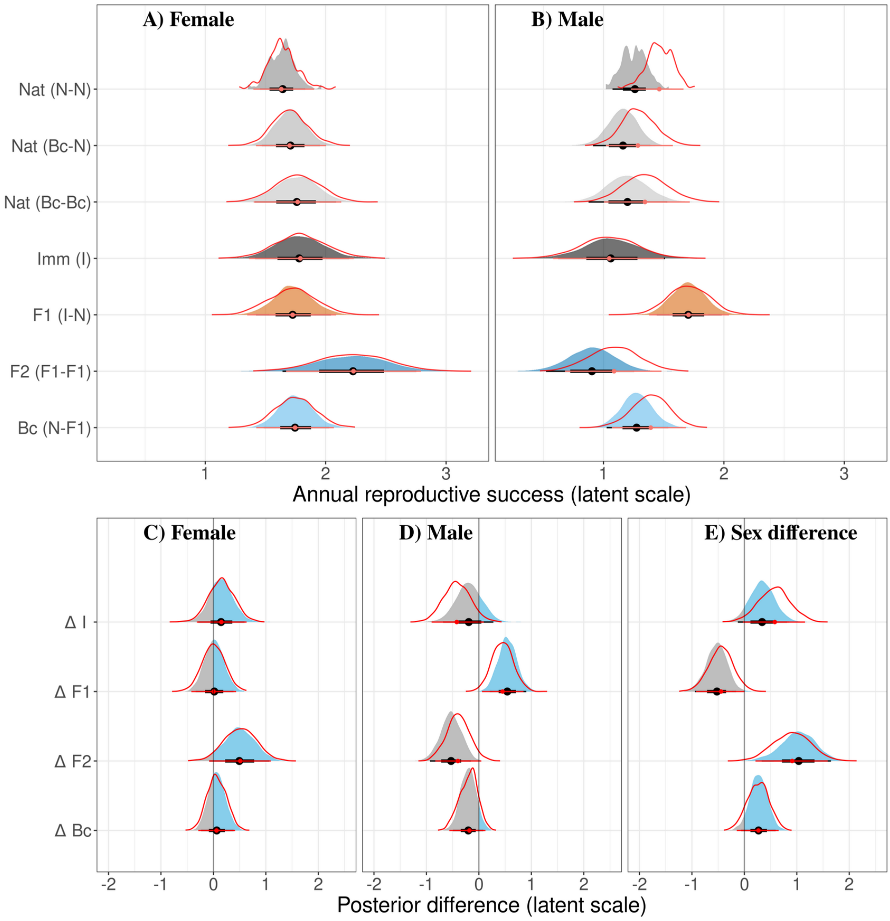


Figure S8.4: Posterior distributions of (A, B) juvenile survival probability for each focal group of individuals, and (C, D, E) planned comparisons between selected groups. Filled posterior distributions show juvenile survival estimated by the model presented in the main manuscript, while the red lines show the posterior distributions of the identical model with sex-specific regressions on *f*. Figure structure and attributes are as in Figure 2. Immigrants are not present at this life stage in the local population. Hence, there is no posterior distribution for immigrants on A and B. C (ΔF1) and D (ΔF2) show the posterior distributions of differences between F1 and natives (ΔF1-NN) and between F2s and µ(F1,NN) (ΔF2-µ(F1,NN)), with no immigrant component.

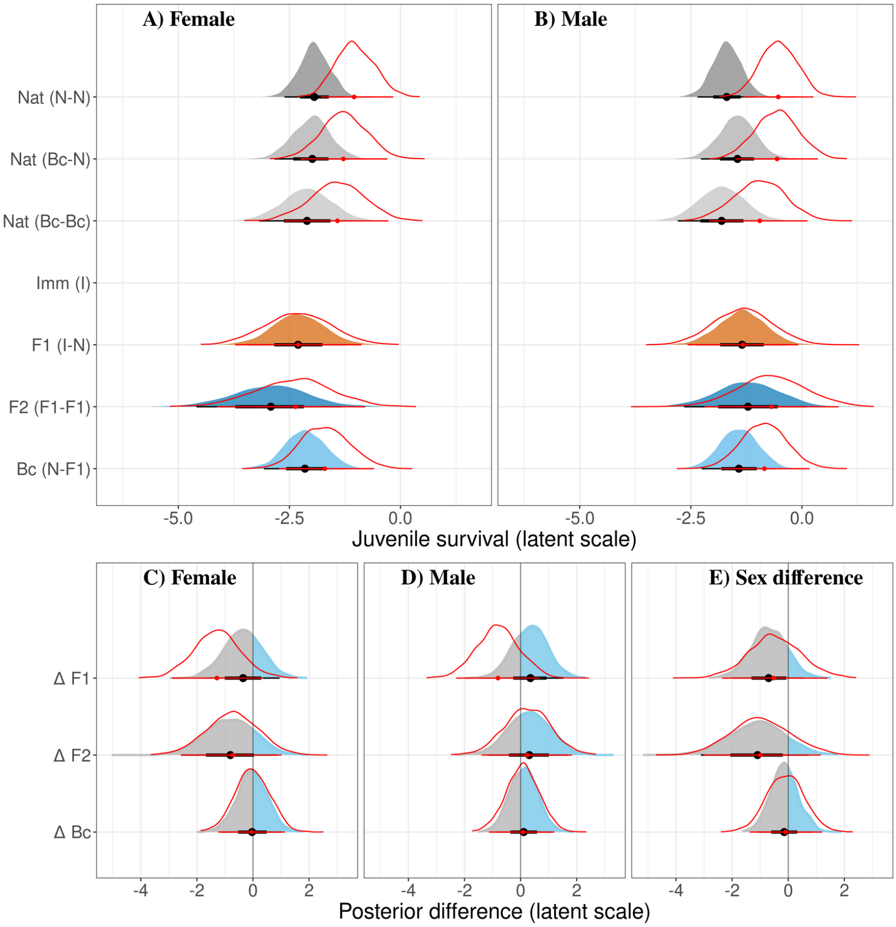
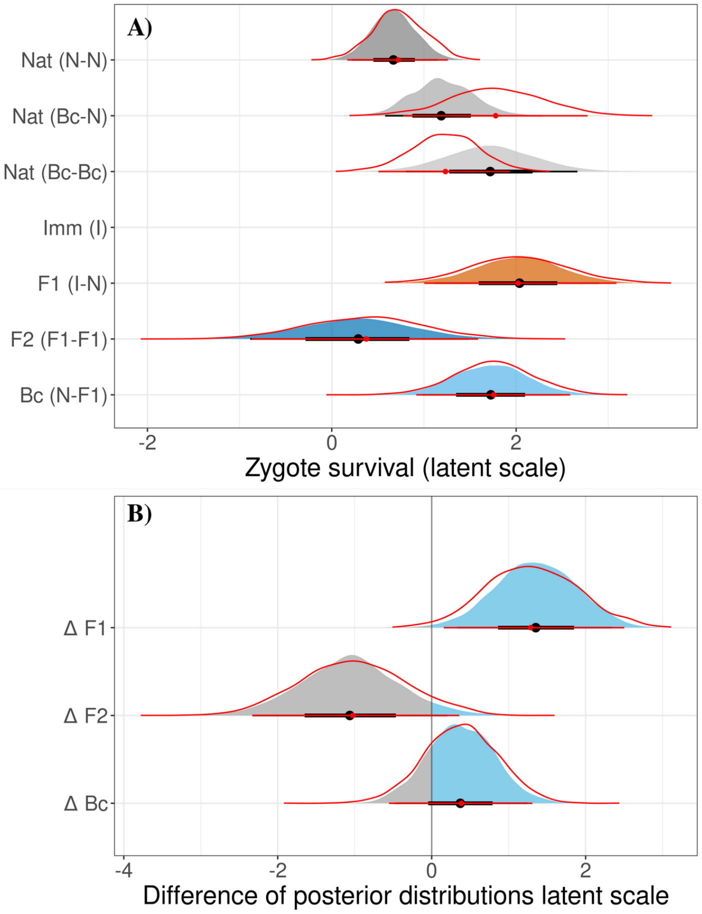


Figure S8.5: Posterior distributions of (A) zygote survival probability for each focal group of clutches and (B) planned comparisons between selected groups. Filled posterior distributions show zygote survival estimated by the model presented in the main manuscript, while the red lines show the posterior distributions of the identical model with a regression on *f*. Figure structure and attributes are as in Figure S8.4 (again with no immigrants), except that females and males are combined.



Literature cited:

Charlesworth, B. 2018. Mutational load, inbreeding depression and heterosis in subdivided populations. Molecular Ecology 27: 4991–5003.

Dickel, L., P. Arcese, P. Nietlisbach, L. F. Keller, H. Jensen, and J. M. Reid. 2021. Are immigrants outbred and unrelated? Testing standard assumptions in a wild metapopulation. Molecular Ecology 30: 5674–5686.

Falconer, D. S., and T. F. C. Mackay, T. F. C. 1996. Introduction to quantitative genetics, 4th ed. Longman, Harlow.

Keller, L. F., J. M. Reid and P. Arcese. 2008. Testing evolutionary models of senescence in a natural population: age and inbreeding effects on fitness components in song sparrows. Proceedings of the Royal Society B 275: 597–604.

Lynch, M. 1991. The Genetic interpretation of inbreeding depression and outbreeding depression. Evolution 45: 622-629.

Lynch, M., and B. Walsh. 1998. Genetics and analyses of quantitative traits. Sinauer Associates, Inc., Sunderland.

Marr, A. B., L. F. Keller, and P. Arcese. 2002. Heterosis and outbreeding depression in descendants of natural immigrants to an inbred population of song sparrows (*Melospiza melodia*). Evolution 56: 131–142.

Mather, K., and J. I. Jinks. 1977. Introduction to Biometrical Genetics. Chapman and Hall, London.

Nietlisbach, P., L. F. Keller, G. Camenisch, F. Guillaume, P. Arcese, J. M. Reid and E. Postma. 2017. Pedigree-based inbreeding coefficient explains more variation in fitness than heterozygosity at 160 microsatellites in a wild bird population. Proceedings of the Royal Society B 284: 20162763.

Reid, J. M., P. Arcese, R. J. Sardell, and L. F. Keller. 2011. Additive genetic variance, heritability, and inbreeding depression in male extra-pair reproductive success. American Naturalist 177(2): 177–187.

Reid, J. M., P. Arcese, P. Nietlisbach, M. E. Wolak, S. Muff, L. Dickel, and L. F. Keller. 2021. Immigration counter-acts local micro-evolution of a major fitness component: Migration-selection balance in free-living song sparrows. Evolution Letters 5: 48–60.

Roff, D. A. and K. Emerson. 2006. Epistasis and dominance: evidence for differential effects in life-history versus morphological traits. Evolution 60: 1981-1990.

Whitlock, M. C., P. K. Ingvarsson, and T. Hatfield. 2000. Local drift load and the heterosis of interconnected populations. Heredity 84: 452–457.

Wolak, M. E., P. Arcese, L. F. Keller, P. Nietlisbach, and J. M. Reid. 2018. Sex-specific additive genetic variances and correlations for fitness in a song sparrow (*Melospiza melodia*) population subject to natural immigration and inbreeding. Evolution 72: 2057–2075.

Paper III

Multi-generation genetic contributions of immigrants reveal cryptic elevated and sex-biased effective gene flow within a natural meta-population

Jane M. Reid[1,2], Lisa Dickel[1], Lukas F. Keller[3,4], Pirmin Nietlisbach[5] & Peter Arcese[6]

[1]Centre for Biodiversity Dynamics, Department of Biology, Norwegian University of Science and Technology (NTNU), Norway. jane.m.reid@ntnu.no, lisa.dickel@ntnu.no

[2]School of Biological Sciences, University of Aberdeen, UK. jane.reid@abdn.ac.uk

[3]Department of Evolutionary Biology & Environmental Studies, University of Zurich, Switzerland. lukas.keller@ieu.uzh.ch

[4]Natural History Museum, University of Zurich, Switzerland.

[5]School of Biological Sciences, Illinois State University, USA. pnietli@ilstu.edu

[6]Department of Forest & Conservation Sciences, University of British Columbia, Canada. peter.arcese@ubc.ca

Running title: Gene flow exceeds immigration

Keywords: Connectivity, dispersal, genealogy, gene drop, gene flow, genetic introgression, heterosis, immigration, meta-population, pedigree.

Statement of Authorship: JMR conceived and undertook the analyses and drafted the manuscript in collaboration with LD. PA oversaw long-term data collection and PN and LFK undertook pedigree construction. All authors contributed substantially to fieldwork, conceptual development and manuscript editing.

Data accessibility: Data and R scripts are available at the Dryad repository: https://doi.org/10.5061/dryad.k6djh9wcv

Abstract

Impacts of immigration on micro-evolution and population dynamics fundamentally depend on net rates and forms of resulting gene flow into recipient populations. Yet, the degrees to which observed rates and sex ratios of physical immigration translate into multi-generational genetic legacies have not been explicitly quantified in natural meta-populations, precluding inference on how movements translate into effective gene flow and eco-evolutionary outcomes. Our analyses of three decades of complete song sparrow (*Melospiza melodia*) pedigree data show that multi-generational genetic contributions from regular natural immigrants substantially exceeded those from contemporary natives, consistent with heterosis-enhanced introgression. However, while contributions from female immigrants exceeded those from female natives by up to three-fold, male immigrants' lineages typically went locally extinct soon after arriving. Both the overall magnitude, and the degree of female bias, of effective gene flow therefore greatly exceeded those which would be inferred from observed physical arrivals, altering multiple eco-evolutionary implications of immigration.

Introduction

Immigration of new individuals into populations can alleviate inbreeding and increase local genetic variation yet disrupt local adaptation, thereby profoundly affecting micro-evolutionary and population dynamic outcomes (Bell et al. 2019; Carlson et al. 2014; Ebert et al. 2002; Lenormand 2002; McDonald & Yeaman 2018; Tallmon et al. 2004). Net effects depend on rates of physical immigration, and on the degree to which immigration translates into successful reproduction and resulting genetic introgression across generations (Garant et al. 2007; Ingvarsson & Whitlock 2000; Peterson et al. 2014; Porter & Benkman 2017; Richardson et al. 2014). Yet, while immigration rates into diverse wild populations have been estimated, and fitness components of natural immigrants versus existing natives have been quantified (e.g. Barbraud & Delord 2021; Martinig et al. 2020; Millon et al. 2019; Mobley et al. 2019; Peterson et al. 2014), net trajectories of immigrants' genetic contributions across multiple subsequent years and generations are rarely explicitly evaluated. The timeframes through which regular natural immigration translates into proportionate or disproportionate effective gene flow among inter-linked sub-populations therefore remain unclear. This precludes holistic understanding of how movement translates into genetic introgression and associated micro-evolution and meta-population dynamics (Bell et al. 2019; Garant et al. 2007; Richardson et al. 2014; Robertson et al. 2018; Saccheri & Brakefield 2002).

Trajectories of introgression following immigration will depend on numerous genetic and environmental effects that together shape the relative reproductive success of immigrants, existing natives, and their descendants (Bell et al. 2019; Fitzpatrick et al. 2016; Grummer et al. 2022; Ingvarsson & Whitlock 2000; Lenormand 2002; Nosil et al. 2005; Rhode & Cruzan 2005; Tallmon et al. 2004; Whiteley et al. 2015). Specifically, new immigrants could have higher or lower local success than natives, depending on balances between genetic effects such as relative heterozygosity and local adaptation alongside proximate ecological and social effects including site familiarity, mate choice and kin interactions (Bonte et al. 2012; Garant et al. 2007; Martinig et al. 2020; Nosil et al. 2005; Peterson et al. 2014; Richardson et al. 2014; Tallmon et al. 2004; Vasudev & Fletcher 2016). Given some degree of population structure, F1 offspring of immigrant-native matings could then have relatively high success due to high heterozygosity and resulting heterosis, increasing effective gene flow (Ebert et al. 2002; Ingvarsson & Whitlock 2000; Tallmon et al. 2004). Conversely, F1s could have low local success due to local maladaptation, parental incompatibilities, and/or heritable dispersal that causes immediate emigration and rapid local loss of recently arrived genetic variants, decreasing effective gene flow (e.g. Doligez & Pärt 2008; Rundle & Whitlock 2001). Any subsequent descendants (e.g. F2 or backcrossed grand-offspring of immigrants) could also have relatively low or high success, including due to outbreeding depression following recombination and epistatic breakdown of co-adapted parental gene complexes, or positive transgenerational effects stemming from heterosis in F1 parents (Frankham et al. 2011; Tallmon et al. 2004). Such negative or positive effects could extend across subsequent generations (Frankham 2016), partly depending on re-accumulation of inbreeding and resulting expression of inbreeding depression in initially successful immigrant lineages (Adams et al. 2011; Bell et al. 2019; Hedrick et al. 2019). Furthermore, such effects could differ between female and male immigrants (Barbraud & Delord 2021; Havird et al. 2016; Martinig et al. 2020; Zajitschek et al. 2009), generating sex-specific genetic legacies that could deviate from the sex ratio of physical immigrants, reshaping the effective degree of sex-specific gene flow among inter-linked sub-populations.

To date, work on wild or recently wild-derived populations showed that key genetic effects on survival and/or reproductive success can be strong, including local adaptation, inbreeding depression, heterosis, outbreeding depression and parent-offspring resemblance in dispersal (Charlesworth & Willis 2009; Doligez & Pärt 2008; Frankham et al. 2011; Hereford 2009; Marr et al. 2002; Nosil et al. 2005; Peterson et al. 2014; Porter & Benkman 2017; Reid et al. 2014; Richards 2000; Richardson et al. 2014; Spigler et al. 2017). Yet, even given estimates of all such effects in any system, net trajectories of introgression following regular natural immigration are still hard to infer. Multiple effects could act simultaneously and sequentially across overlapping generations, with sex-specific impacts that could also depend on immigration rates, mate choice, and forms of demographic and environmental variation that shape population dynamics

(Cheptou & Donohue 2011; Grummer et al. 2022; Vasudev & Fletcher 2016). Hence, one incisive approach is to directly estimate immigrants' genetic contributions to recipient populations across multiple years and generations following arrival by counting genealogical descendants (Åkesson et al. 2016; Chen et al. 2019; Saccheri & Brakefield 2002). Such analyses capture net temporal dynamics of lineage introgression or extinction, thereby revealing the degree to which observed rates and sex ratios of physical immigration predict magnitudes of effective sex-specific gene flow in nature.

To enact this approach, expected genetic contributions of any focal immigrant or native individual to a focal population in any year following arrival or recruitment can be calculated from complete long-term pedigree data (Adams et al. 2011; Barton & Etheridge 2011; Chen et al. 2019; Reid et al. 2019; Saccheri & Brakefield 2002). Most simply, the probability that any (hypothetical) allele present in any focal individual will be present identical-by-descent in each individual conceived in any subsequent cohort can be computed, then summed to give the focal individual's total expected genetic contribution given its observed pedigree of descendants. Further, stochastic 'gene-drop' simulations can be implemented to capture variation stemming from Mendelian sampling (i.e. genetic drift), and estimate probabilities of local allele extinction (Supporting Information S2, Chen et al. 2019; Hunter et al. 2019; Reid et al. 2019). Yet, while substantial pedigree data are accumulating for diverse wild populations, multi-generation genetic contributions resulting from regular natural immigrants versus contemporary natives have rarely been quantified. This precludes any general synthesis on how physical movement translates into short-term and longer-term dynamics of introgression in structured (meta-)populations.

A song sparrow (*Melospiza melodia*) population inhabiting X̱OX̱ DEȽ (English: Mandarte island), BC, Canada, exhibits multiple genetic effects that must affect the dynamics and net degree of introgression. Briefly, Mandarte is one of numerous local islands and habitat patches that hold song sparrows, and receives regular immigrants of both sexes (approximately 1 immigrant/year on average, Dickel et al. 2021; Reid et al. 2021; Wilson & Arcese 2008). These immigrants introduce new genetic variation (Keller et al. 2001), prevent local coefficients of inbreeding f and resulting expression of inbreeding depression from increasing (current mean f≈0.08, Keller 1998; Reid et al. 2014, 2021), and alter the mating system by shaping patterns of extra-pair reproduction and inbreeding (Reid et al. 2006; Reid & Arcese 2020; Wolak & Reid 2016). Yet, while immigration causes heterosis manifested as high fitness of F1 offspring, outbreeding depression emerges in F2 grand-offspring (Dickel et al. 2024; Marr et al. 2002). Furthermore, genetic contributions from immigrants have additive effects that decrease local juvenile survival (due to increased mortality and/or emigration, Reid et al. 2021). The net consequences of all such effects for the trajectory and ultimate degree of genetic introgression are intrinsically complex, and have not been quantified in any wild (meta-)population experiencing regular natural immigration.

Accordingly, we use three decades of complete song sparrow pedigree data to quantify net trajectories of genetic contributions of natural immigrants to the focal population. Specifically, we test whether arriving immigrants ultimately make greater, similar or smaller expected genetic contributions than contemporary natives, and quantify the timeframes over which such outcomes emerge. Further, we test whether the trajectories and endpoints of introgression differ between females and males. We demonstrate strongly elevated and sex-biased effective gene flow into the focal population, of magnitudes that substantially exceed those which would be inferred from observed physical immigration. We highlight key genetic causes and evolutionary implications of these outcomes, which reshape the net degree of sex-specific introgression.

Methods

Pedigree data

Each breeding season (April-August) since 1975, all song sparrow breeding attempts on Mandarte (6 hectares, latitude 48.6329°, longitude −123.2859°) were monitored. All chicks surviving to six days post-hatch were individually colour-ringed (Keller 1998; Reid et al. 2014). Newly arriving immigrants, initially identified as unringed adults, were mist-netted and colour-ringed soon after arriving (Dickel et al. 2021; Marr et al. 2002; Reid et al. 2006, 2021). Social parents of all chicks were identified from colour-rings, with sexes attributed by female incubation and male song. Since 1993, genetic parentage was assigned with very high statistical confidence using microsatellite genotypes (revealing 28% extra-pair paternity, Nietlisbach et al. 2017; Reid et al. 2014; Sardell et al. 2010). These analyses also confirmed that presumed immigrants (i.e. initially unringed adults) were effectively unrelated to the local population at arrival, and hence were true immigrants (Dickel et al. 2021). A complete pedigree spanning 1975-2019 was compiled, with full genetic verification since 1993, and additional genetic verification of parentage of adults breeding during 1989-1992 (Nietlisbach et al. 2017; Reid et al. 2014; Sardell et al. 2010). Data collection was approved by University of British Columbia Animal Care Committee with ringing permits from Environment and Climate Change Canada.

Genetic contributions

To compare expected genetic contributions, we identified new immigrants that arrived each spring and natives that recruited (i.e. were age one year) simultaneously. Here, we defined natives as Mandarte-hatched individuals with four Mandarte-hatched grandparents, thereby excluding F1, F2 and backcross descendants of previous immigrants. Focal immigrants and natives were therefore matched for the timing of first potential reproduction on Mandarte (and matched for age assuming that immigrants are one year old at arrival). Analyses were restricted to individuals recruited during 1990-2012 (presumed hatched 1989-2011). This timeframe encompasses the cohorts for which all individuals' descendant genealogies have been genetically verified for ≥8 years up to 2019, and spans approximately three generations given the mean generation time of approximately 2.5 years (Reid et al. 2019).

We initially used pedigree algorithms to calculate the probability that any (hypothetical) allele present in any focal immigrant or native will be present identical-by-descent in any subsequent individual. Summing these contributions across all chicks ringed in each year gives each focal individual's total expected genetic contribution (Tot_{EGC}) to each cohort, starting from its recruitment year (termed year 1, Supporting Information S1,S2). However, an individual's total realised genetic contribution (Tot_{RGC}) at any locus can deviate from Tot_{EGC}, even without selection on any genetic variant, due to Mendelian sampling of alleles across generations and resulting drift. Further, while stabilised Tot_{EGC} predicts medium-term probabilities of local allele persistence versus extinction (given low immigration rates, Barton & Etheridge 2011), short-term allele extinction probabilities vary non-linearly with Tot_{EGC}, with variation stemming from the pedigree structure of focal individuals' descendants (Reid et al. 2019). Such outcomes cannot be readily calculated analytically for complex pedigrees. We therefore used 'gene-drop' simulations to estimate the total realised number of copies of a (hypothetical) allele initiated in any focal immigrant or native that is present in any subsequent cohort (Tot_{RGC}), and estimate the probability of local allele extinction within the observed timeframe (P_0).

Specifically, each focal immigrant and native was assigned a unique allele identity, which was 'dropped' down the pedigree assuming autosomal Mendelian inheritance (Supporting Information S2). The identities of alleles inherited by all chicks in each subsequent cohort were extracted and frequencies computed, giving each focal individual's $\mathrm{Tot}_{\mathrm{RGC}}$. N=1000 replicate gene-drops were implemented, generating a distribution of stochastic outcomes. Mean $\mathrm{Tot}_{\mathrm{RGC}}$ across replicates (hereafter $\mu\mathrm{Tot}_{\mathrm{RGC}}$) approximates $\mathrm{Tot}_{\mathrm{EGC}}$, and is interpretable as an individual's expected genetic contribution across numerous unlinked loci. The number of instances (N_0) of zero copies of each focal individual's unique allele in each successive cohort was extracted. Extinction probability for each allele in each cohort was computed as $P_0=N_0/N$, where $P_0=1$ implies certain lineage extinction. Such gene-drop simulations envisage neutral or weakly selected alleles, effectively assuming that fitness is shaped by numerous small genetic effects, as increasingly empirically supported for diverse traits (Grummer et al. 2022; Sella & Barton 2019). They encompass genetic contributions arising through inter-breeding (and inbreeding) descendants of both sexes, and introgression among initially distinct immigrant and native lineages. Pedigree analyses were implemented using package nadiv (Wolak 2012) in R v4.2.1 (R Core Team 2022).

Data and analysis structures

In total, 25 new immigrants arrived on Mandarte during 1990-2012, involving 13 different years (Figure 1). Overall, 249 defined natives recruited in these same years (110 females, 139 males, Figure 1). Mean local population size was 73±30SD adults (median 62, range 24-128, Supporting Information S4). Neither the absolute number of new immigrants per year, nor the proportion of new recruits that were immigrants, changed substantively across years (Figure 1, Supporting Information S4). Immigration was not strongly sex-biased overall, with 14 female and 11 male immigrants (56%:44%, which does not differ significantly from 50:50, $\chi_1^2=0.4$, Monte-Carlo $p\approx0.69$). However, the sex ratio of arriving immigrants changed during 1990-2012, from predominantly females (13 of 14 females arrived before 2006) to predominantly males (8 of 11 males arrived since 2008, Figure 1, binomial regression of sex on year $\beta=-0.14$, 95%CI -0.27 – -0.03, $p=0.023$). Because of this data structure, we undertook two sets of analyses.

First, for immigrants and natives of both sexes that recruited during 1990-2012, we extracted each individual's gene-dropped Tot_{RGC} to each of the eight cohorts of chicks ringed following each individual's recruitment (i.e. recruitment year to seven years later). We fitted generalised linear (mixed) models (GLMs) to test whether Tot_{RGC} varied with individual status (immigrant versus native), sex and the status-by-sex interaction. Second, to consider the longer available timeframe for female immigrants and natives recruited during 1990-2005, we extracted their Tot_{RGC} to each of the fifteen cohorts of chicks ringed following recruitment, and fitted further GLMs to test whether Tot_{RGC} differed between immigrants and natives.

Here, we fitted separate GLMs for Tot_{RGC} in each year from arrival, generating eight sets of models for the shorter-term analyses of both sexes, and fifteen sets of models for the longer-term analyses of females. We assumed Poisson error structures (since $\mathrm{Tot}_{\mathrm{RGC}}$ is count data) with log link. Since population size varied among years (Supporting Information S4), the total number of chicks ringed in each cohort was modelled as an offset variable. Realised genetic contributions are therefore effectively estimated as proportions of the total possible (hereafter $\mathrm{Prop}_{\mathrm{RGC}}$). For the shorter-term analyses, natives of both sexes were retained for each year in which at least one immigrant of either sex arrived. Random recruitment year effects were fitted to account for variation due to population conditions (e.g. adult sex ratio, which affects mean reproductive success of males). The longer-term analyses were restricted to female immigrants and contemporary female natives. Separate GLMs were fitted to each set of values of $\mathrm{Tot}_{\mathrm{RGC}}$ obtained from each of the N=1000 gene-drops. Mean effects were computed across the N replicates, weighted by the inverse square of the standard errors. Mean 95% confidence intervals were computed to illustrate associated uncertainty. This modelling process therefore encompasses variation due to Mendelian sampling alongside uncertainty inherent in estimation of effects. GLMs were fitted and estimates extracted using R packages lme4 and emmeans (Bates et al. 2015; Lenth 2022). Supporting Information S3 summarises further details of analytical decisions and interpretations, including minor adjustments to facilitate model convergence.

Finally, to reveal underlying variation, we summarised individuals' values of $\mu\mathrm{Tot}_{\mathrm{RGC}}$ and P_0 in years eight and fifteen from immigrants' arrivals for the datasets on both sexes and females only respectively. To account for variation in cohort sizes, we divided $\mu\mathrm{Tot}_{\mathrm{RGC}}$ by the total number of chicks ringed in the relevant year, giving proportional values (μProp_{RGC}). Abbreviations are summarised in Supporting Information S1.

Results

Across the eight-year dataset comprising both sexes (totalling 25 immigrants and 249 natives), Prop_{RGC} from immigrants exceeded that from natives to an increasing degree across the years following immigrants' arrivals (Figure 2). The increase occurred because Prop_{RGC} from immigrants tended to increase across years, while that from natives did not (Figure 2). Hence, by years 6-8 from recruitment, Prop_{RGC} from immigrants was almost double that from contemporary natives (Figure 2). Effective gene flow into the recipient population was therefore substantially greater than would be simply inferred from the observed frequency of immigration.

Further, overall immigrant-native differences in Prop_{RGC} (Figure 2) were underpinned by strong sex-specific effects. Across all immigrants and natives combined, Prop_{RGC} for females exceeded that for males (Figure 3A). Sex-specific comparisons of immigrants versus natives then showed that Prop_{RGC} from female immigrants typically exceeded that from contemporary female natives (Figure 3B). Effect sizes were substantial: 5-8 years from recruitment, Prop_{RGC} from female immigrants was 2-3 times that from natives (Figure 3B). In contrast, Prop_{RGC} from male immigrants was typically lower than male natives, although the 95% confidence intervals for the differences were often wide and spanned or approached zero (Figure 3C).

Hence, there was a strong status-by-sex interaction, especially after four years from recruitment, showing that the higher Prop_{RGC} from female immigrants versus natives substantially exceeded any difference in Prop_{RGC} between male immigrants and natives (Figure 3D). Consequently, coupled with the higher overall mean Prop_{RGC} from females versus males (Figure 3A), individual female immigrants on average made up to four-fold greater genetic contributions to the focal population than individual male immigrants across the years following recruitment (Figures 3B,C).

Examining individuals' values of $\mu\text{Prop}_{\text{RGC}}$ eight years from recruitment showed that, of the eleven immigrant males, nine had values of zero at that time (Figure 4B, i.e. zero descendants). Since their lineages were already locally extinct (i.e. $P_0=1$), these males could not make any subsequent local genetic contribution. Hence, there were only two male immigrants with potential for lasting legacies, one whose $\mu\text{Prop}_{\text{RGC}}$ after eight years was substantial, and one whose $\mu\text{Prop}_{\text{RGC}}$ was very small (Figure 4B). In contrast, 11 of 14 immigrant females had non-zero $\mu\text{Prop}_{\text{RGC}}$ (including five individuals with values ≥5%), which could consequently make longer-term contributions (Figure 4A). Consequently, the probability of having zero $\mu\text{Prop}_{\text{RGC}}$ after eight years was higher in immigrant males than immigrant females (9 of 11 versus 3 of 14, $\chi_1^2=9.0$, Monte-Carlo $p\approx0.006$). It was also lower in immigrant females than native females (3 of 14 versus 62 of 110, $\chi_1^2=6.1$, $p\approx0.02$), but did not differ between immigrant and native males (9 of 11 versus 90 of 139, $\chi_1^2\approx1.3$, $p\approx0.33$). As expected, the probability that an allele originating in a focal individual would be locally extinct after eight years (P_0) decreased non-linearly with increasing $\mu\mathrm{Prop}_{\mathrm{RGC}}$ (Figure 4C). Nevertheless, differences in P_0 between immigrant and native females and males mirrored those for $\mu\mathrm{Prop}_{\mathrm{RGC}}$. Specifically, local allele extinction probabilities were higher for immigrant males than immigrant females and lower for immigrant females than native females, with no difference between immigrant and native males (Supporting Information S5).

The longer-term female-only dataset spanning 15 years from recruitment (totalling 13 immigrants and 60 natives that recruited in 9 years) further showed that female immigrants had substantially higher $\mathrm{Prop}_{\mathrm{RGC}}$ than natives (Figure 5A). The approximately 2.5-fold difference was broadly stable through years 5-15 from immigrants' arrivals, even though the means of both groups decreased slightly across this timeframe (Figure 5A, as expected with ongoing immigration). Examining $\mu\mathrm{Prop}_{\mathrm{RGC}}$ in year 15 showed that natives tended to be more likely than immigrants to have values of zero at that time (31 of 60 natives versus 3 of 13 immigrants, $\chi^2_1=3.5$, Monte-Carlo $p\approx0.07$). P_0 decreased approximately linearly with $\mu\mathrm{Prop}_{\mathrm{RGC}}$ across the observed range of variation (Figure 5B), and hence was lower for immigrants than natives (Supporting Information S5).

Discussion

Gene flow resulting from immigration shapes local genetic variation, adaptation, inbreeding and heterosis; yet effective gene flow also depends on these attributes, and hence on manifold genetic effects underlying the fitness of immigrants, natives, and their descendants (Garant et al. 2007;

Grummer et al. 2022; Ingvarsson & Whitlock 2001; Peterson et al. 2014; Porter & Benkman 2017). Such compound genetic effects, intertwined with environmental effects and drift, could generate complex multi-generation trajectories of lineage introgression versus extinction. But, such outcomes have not been quantified in wild populations receiving regular natural immigrants (Bell et al. 2019; Richardson et al. 2014). Our analyses of comprehensive song sparrow pedigree data revealed substantially greater per capita genetic contributions from immigrants than contemporary natives across years (and hence generations) following immigrants' arrivals. Further, there were strong sex-specific effects where female immigrants' contributions substantially exceeded those of natives, while most male immigrants' lineages were already locally extinct soon after arriving. Net effective gene flow was consequently elevated, and much more strongly female-biased, than implied by observed physical immigration, altering the magnitude, form and eco-evolutionary implications of introgression.

Elevated gene flow

The higher Prop_{RGC} from individual immigrants versus natives emerged over ≥5 years from immigrants' arrivals (Figure 2), representing approximately two generations. This timeframe matches theory predicting that heterosis in F1 offspring of immigrant-native matings can increase effective immigration rates and resulting effective gene flow (Ingvarsson & Whitlock 2000). Here, high F1 reproductive success generates numerous grand-offspring of immigrants, enhancing the representation of immigrants' genes in recipient populations, even if immigrants themselves do not have notably high success. Such effects are predicted to be greatest, increasing effective gene flow up to 5-10 fold, given small effective size of the recipient population, weak to moderate selection coefficients against numerous largely recessive deleterious alleles, and sparse gene flow (≤1 effective immigrant per generation, Ingvarsson & Whitlock 2000). These conditions likely approximately apply in our system, where lifetime reproductive success (i.e. total chicks produced) does not differ markedly between immigrants and natives overall, but strong inbreeding depression, and heterosis manifested as increased F1 survival and reproductive success, is evident (Supporting Information S6, Dickel et al. 2024; Keller 1998; Marr et al. 2002; Nietlisbach et al. 2017; Reid et al. 2014). The high Prop_{RGC} of immigrants versus natives then implies that heterosis, which can substantially reflect genetic dominance effects stemming from among-population drift (Charlesworth & Willis 2009; Ingvarsson & Whitlock 2000), outweighs known low fitness of F2 descendants resulting from F1-F1 matings (consistent with epistatic breakdown, Dickel et al. 2024), and negative additive genetic effects of immigrants' genes on local juvenile survival (Reid et al. 2021). Heterosis-enhanced introgression could consequently be strong enough to cause local maladaptation.

Despite the central importance of effective gene flow in shaping micro-evolution and population outcomes, multi-generation trajectories of introgression following regular natural immigration within weakly inbred meta-populations have not previously been explicitly quantified (Ingvarsson

& Whitlock 2000; Saccheri & Brakefield 2002; Whiteley et al. 2015). Experimental insertions of differentiated individuals into artificial inbred populations showed that immigrants' genetic contributions can substantially exceed those of defined natives over one or multiple generations, consistent with heterosis-enhanced introgression (e.g. *Daphnia magna*, Ebert et al. 2002; *Silene alba*, Richards 2000; *Bicyclus anynana*, Saccheri & Brakefield 2002). Fitness components of natural or translocated immigrants into highly inbred wild populations, and of their offspring, can also be high, potentially generating elevated initial genetic introgression and population growth ('genetic rescue', e.g. *Canis lupus*, Adams et al. 2011, Åkesson et al. 2016; *Passer domesticus*, Alif et al. 2022; *Poecilia reticulata*, Fitzpatrick et al. 2016; *Petroica australis*, Heber et al. 2013; *Ovis canadensis*, Hogg et al. 2006). Yet, effective gene flow between more strongly diverged populations or ecotypes, as commonly quantified in studies on local adaptation and incipient speciation, can be substantially less than expected from observed physical immigration, reflecting ecological and/or genetic incompatibilities manifested through reduced viability and/or fecundity (Garant et al. 2007; Nosil et al. 2005; Porter & Benkman 2017; e.g. *Onchorhynchus nerka*, Peterson et al. 2014; *Belonocnema treatae*, Zhang et al. 2021). Meanwhile, in Florida scrub jays (*Aphelocoma coerulescens*), large cohorts of immigrants made substantial cumulative expected genetic contributions to future generations, but trajectories of individual immigrants' versus natives' contributions were not explicitly compared (Chen et al. 2019). Our study fills an important gap across this spectrum of biological divergence, showing that regular natural immigration into a weakly inbred (sub-)population can induce substantially elevated effective gene flow, that is not fully manifested until several years after immigrants' arrivals.

Sex-specific gene flow

Given the paucity of studies that explicitly quantify multi-generation trajectories of introgression following regular natural immigration, there is inevitably little understanding of how such trajectories can differ between females and males, generating effective sex-biased introgression. In song sparrows, $\mathrm{Prop}_{\mathrm{RGC}}$ from female immigrants substantially exceeded that from male immigrants (Figure 3). Yet, inferring sex-specific effects requires care given that the sex ratio of immigrants changed across years (Figure 1). Apparent sex-specific effects could consequently reflect temporal effects, if contributions from immigrants of both sexes changed across years. However, there is no evidence of such changes. Examining values of $\mu\mathrm{Prop}_{\mathrm{RGC}}$ in relation to immigrants' arrival years showed that the most recent considered female immigrant (arrived 2008) had substantial $\mu\mathrm{Prop}_{\mathrm{RGC}}$, especially after eight years, with little or no evidence that values decreased across years (Figure 6). Further, the two male immigrants that arrived earliest (in 1990 and 1991) had very small or zero $\mu\mathrm{Prop}_{\mathrm{RGC}}$, as did the recent male immigrants that arrived since 2008 (Figure 6). The inference that expected genetic contributions of immigrants are sex-specific, rather than solely year-specific, is therefore robust.

The overall sex-specific effects on $\mathrm{Prop}_{\mathrm{RGC}}$ (lower in males, Figure 3A) reflect the structure of male reproductive success, including that F1 sons of immigrant-native pairings have high mean success (Supporting Information S6, Dickel et al. 2024) but are not included as focal individuals in current analyses (Supporting Information S3). The status-by-sex interactions (Figures 3B-D) must then reflect sex-specific variation in success of immigrants and/or their offspring. Indeed, reproductive success tends to be lower for male immigrants than natives, partly reflecting relatively low mating success of immigrants, but if anything slightly higher for female immigrants than natives (Supporting Information S6, Dickel et al. 2024). While these trends are only weakly statistically supported, the at least average success of female immigrants can seed escalations through F1 heterosis, but heterosis cannot increase $\mathrm{Prop}_{\mathrm{RGC}}$ for male immigrants that produce no F1 offspring. Low reproductive success of male immigrants has been documented elsewhere, for example following experimental crosses in *Pomatoschistus minutus* (Svensson et al. 2017) and natural immigration in three long-lived seabird species (Barbraud & Delord 2021). In contrast, male immigrants had high success in *Tamiasciurus hudsonicus* (Martinig et al. 2000), while introduction of genetically distinct *Mus domesticus* into an isolated population culminated in elevated Y-chromosome introgression, likely reflecting high mating success of introduced males (Jones et al. 1995).

Irrespective of the exact reproductive constraints, the notable outcome is that effective gene flow into the focal song sparrow population was substantially female-biased. $\mathrm{Prop}_{\mathrm{RGC}}$ for female immigrants greatly exceeded that for male immigrants. Correspondingly, most female immigrants had non-zero probabilities of medium-term genetic contributions, while most male immigrants left zero genetic legacy (Figures 3-5). Such strong sex-bias in effective gene flow is not evident from the sex ratio of physical immigrants, which did not differ substantively from 50:50. Future studies aiming to quantify and understand sex-specific gene flow in diverse systems should therefore consider multi-generational survival and reproductive success of immigrants, natives and their descendants (e.g. Nosil et al. 2005; Porter & Benkman 2017; Robertson et al. 2018). Simply recording the relative frequency and sex of arriving immigrants may be insufficient, also implying that direct molecular genetic estimates of sex-specific gene flow may not accurately reflect rates of sex-specific physical movement.

Eco-evolutionary implications

Our evidence of cryptic elevated and strongly sex-biased effective gene flow has important eco-evolutionary implications. In general, the scope for sex-specific ‘genetic rescue’ has scarcely been considered, either theoretically or empirically (Gemmell et al. 2004; Zajitschek et al. 2009). Given that immigrant song sparrows likely originate from diverse populations (Dickel et al. 2021), elevated female-biased gene flow could increase local mitochondrial genetic variation. This could alleviate any accumulation of mitochondrial mutations, including expected accumulations of male-detrimental mutations, and thereby increase fitness in one or both sexes

(Gemmell et al. 2004), at least in the absence of overarching mito-nuclear incompatibilities (e.g. Havird et al. 2016).

Such outcomes could feed back to shape evolution of sex-biased dispersal, which presumably reflects multifaceted sex-specific costs and benefits of departure, movement and settlement (Bonte et al. 2012; Doligez & Pärt 2008; Li & Kokko 2019). Net sex-specific selection on dispersal cannot be evaluated solely by quantifying fitness of immigrants in recipient populations (i.e. without data on fitness of residents in origin populations). Nevertheless, the fact that local $\mathrm{Prop}_{\mathrm{RGC}}$ from female immigrants substantially exceeded that from natives, while $\mathrm{Prop}_{\mathrm{RGC}}$ from male immigrants was often zero, implies a (multi-generational) component of sex-specific selection on dispersal manifested as sex-specific legacies of settled emigrants. This component, shaped by combinations of additive and non-additive genetic effects acting in immigrants' descendants, should be explicitly factored into empirical and theoretical treatments of evolution of sex-biased dispersal.

Further, the higher $\mathrm{Prop}_{\mathrm{RGC}}$ of female versus male immigrants (Figure 3B,C), coupled with the changing sex ratio of new immigrants from female-biased to male-biased across years (Figure 1), implies that effective introgression of incoming genetic variants into the focal song sparrow population decreased somewhat, even with no marked change in the overall physical immigration rate. Indeed, trends towards decreasing $\mu\mathrm{Prop}_{\mathrm{RGC}}$ per immigrant are evident across the observed timeframe (Figure 6). The shift from female-biased immigration towards male-biased immigration presumably reflects changing sex-specific demography and/or ecology across the meta-population (Wilson & Arcese 2008). Indeed, sex-specific density-dependence in dispersal can occur (Bonte et al. 2012). Any ongoing shift towards male-biased immigration could therefore cause a cryptic decrease in effective gene flow, effectively genetically decoupling sub-populations despite ongoing physical movement.

Overall, our demonstrations that effective gene flow is cryptically female-biased, coupled with changing immigrant sex ratios, imply that our focal population is at demographic and genetic disequilibrium. Such disequilibria can substantially alter standard eco-evolutionary predictions, for example reversing expected relationships among population size, drift, heterozygosity and inbreeding depression (Spigler et al. 2017). Accordingly, our key result that the magnitude and sex-bias of effective gene flow within a natural meta-population can be substantially decoupled from physical movement should now be incorporated into efforts to predict the impacts of dispersal and resulting immigration on eco-evolutionary dynamics and population persistence. This will be best achieved through non-equilibrium genetically explicit and/or quantitative genetic models that capture interacting demographic, genetic and ecological effects acting within and across generations. Such approaches could reveal the degree to which heterosis-enhanced gene flow could drive elevated introgression of locally maladaptive alleles, ultimately decreasing mean individual and population fitness despite initial beneficial effects of immigration.

Acknowledgements

We thank the Tsawout and Tseycum First Nations Bands for allowing access to X̱OX̱ DEŁ (Mandarte); everyone who contributed to long-term data collection; NSERC (Canada), the Swiss National Science Foundation, the Research Council of Norway (SFF-III, project 223257) and NTNU for funding; and Bob O’Hara for helpful discussions on analyses.

References

Adams, J.R., Vucetich, L.M., Hedrick, P.W., Peterson, R.O. & Vucetich, J.A. (2011). Genomic sweep and potential genetic rescue during limiting environmental conditions in an isolated wolf population. *Proc. R. Soc. B* 278, 3336-3344.

Åkesson, M., Liberg, O., Sand, H., Wabakken, P., Bensch, S. & Flagstad, Ø. (2016). Genetic rescue in a severely inbred wolf population. *Mol. Ecol.* 25, 4745-4756.

Alif, Ž., Dunning, J., Chik, H.Y.J., Burke, T. & Schroeder, J. (2022). What is the best fitness measure in wild populations? A case study on the power of short-term fitness proxies to predict reproductive value. *PLoS One* 17, e0260905.

Barbraud, C. & Delord, K. (2021). Selection against immigrants in wild seabird populations. *Ecol. Let.* 24, 84-93.

Barton, N.H. & Etheridge, A.M. (2011). The relation between reproductive value and genetic contribution. *Genetics* 188, 953-973.

Bates D., Mächler, M., Bolker, B. & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Soft.* 67, 1-48.

Bell D.A., Robinson, Z.L., Funk, W.C., Fitzpatrick, S.W., Allendorf, F.W., Tallmon, D.A. & Whiteley, A.R. (2019). The exciting potential and remaining uncertainties of genetic rescue. *Trends Ecol. Evol.* 34, 1070-1079.

Bonte, D., van Dyck, H., Bullock, J.M., Coulon, A., Delgado, M., Gibbs, M., *et al.* (2012). Costs of dispersal. *Biol. Rev.* 87, 290-312.

Carlson, S.M., Cunningham, C.J. & Westley, P.A.H. (2014). Evolutionary rescue in a changing world. *Trends Ecol. Evol.* 29, 521-530.

Charlesworth, D. & Willis, J.H. (2009). The genetics of inbreeding depression. *Nat. Rev. Gen.* 10, 783-796.

Chen, N., Juric, I., Cosgrove, E.J., Bowman, R., Fitzpatrick, J.W., Schoech, S.J., *et al.* (2019). Allele frequency dynamics in a pedigreed natural population. *Proc. Natl. Acad. Sci.* 116, 2158-2164.

Cheptou, P.O. & Donohue, K. (2011). Environment-dependent inbreeding depression: its ecological and evolutionary significance. *New Phytol.* 189, 395-407.

Dickel, L., Arcese, P., Nietlisbach, P., Keller, L.F., Jensen, H. & Reid, J.M. (2021). Are immigrants outbred and unrelated? Testing standard assumptions in a wild metapopulation. *Mol. Ecol.* 30, 5674-5686.

Dickel, L., Arcese, P., Keller, L.F., Nietlisbach, P., Goedert, D., Jensen, H. & Reid, J.M. (2024). Multi-generational fitness effects of natural immigration indicate strong heterosis and epistatic breakdown in a wild bird population. *Am. Nat.,* in press.

Doligez, B. & Pärt, T. (2008). Estimating fitness consequences of dispersal: a road to ‘know-where’? Non-random dispersal and the underestimation of dispersers’ fitness. *J. Anim. Ecol.* 77, 1199-1211.

Ebert, D., Haag, C., Kirkpatrick, M., Riek, M., Hottinger, J. & Pajunen, V.I. (2002). A selective advantage to immigrant genes in a *Daphnia* metapopulation. *Science* 295, 485-488.

Fitzpatrick, S.W., Gerberich, J.C., Angeloni, L.M., Bailey, L.L., Broder, E.D., Torres-Dowdall, J., *et al.* (2016). Gene flow from an adaptively divergent source causes rescue through genetic and demographic factors in two wild populations of Trinidadian guppies. *Evol. Appl.* 9, 879-891.

Frankham, R., Ballou, J.D., Eldridge, M.D.B., Lacy, R.C., Ralls, K., Dudash, M.R. & Fenster, C.B. (2011). Predicting the probability of outbreeding depression. *Cons. Biol.* 25, 465-475.

Frankham, R. (2016). Genetic rescue benefits persist to at least the F3 generation, based on a meta-analysis. *Biol. Cons.* 195, 33-36.

Garant, D., Forde, S.E. & Hendry, A.P. (2007). The multifarious effects of dispersal and gene flow on contemporary adaptation. *Func. Ecol.* 21, 434-443.

Gemmell, N.J., Metcalf, V.J. & Allendorf, F.W. (2004). Mother’s curse: the effect of mtDNA on individual fitness and population viability. *Trends Ecol. Evol.* 19, 238-244.

Grummer, J.A., Booker, T.R., Matthey-Doret, R., Nietlisbach, P., Thomaz, A.T. & Whitlock, M.C. (2022). The immediate costs and long-term benefits of assisted gene flow in large populations. *Cons. Biol.* 36, e13911.

Havird, J.C., Fitzpatrick, S.W., Kronenberger, J., Funk, W.C., Angeloni, L.M. & Sloan, D.B. (2016). Sex, mitochondria and genetic rescue. *Trends Ecol. Evol.* 31, 96-99.

Heber, S., Varsani, A., Kuhn, S., Girg, A., Kempenaers, B. & Briskie, J. (2013). The genetic rescue of two bottlenecked South Island robin populations using translocations of inbred donors. *Proc. R. Soc B* 280, 20122228.

Hedrick, P.W., Robinson, J.A., Peterson, R.O. & Vucetich, J.A. (2019). Genetics and extinction and the example of Isle Royale wolves. *Anim. Cons.* 22, 302-309.

Hereford, J. (2009). A quantitative survey of local adaptation and fitness trade-offs. *Am. Nat.* 173, 579-588.

Hogg, J.T., Forbes, S.H., Steele, B.M. & Luikart, G. (2006). Genetic rescue of an insular population of large mammals. *Proc. R. Soc. B* 273, 1491-1499.

Hunter, D.C., Pemberton, J.M., Pilkington, J.G. & Morrissey, M.B. (2019). Pedigree-based estimation of reproductive value. *Heredity* 110, 433-444.

Ingvarsson, P.K. & Whitlock, M.C. (2000). Heterosis increases the effective migration rate. *Proc. R. Soc. B.* 267, 1321-1326.

Jones, C.S., Noble, L.R., Jones, J.S., Tegelström, H., Triggs, G.S. & Berry, R.J. (1995). Differential male genetic success determines gene flow in an experimentally manipulated mouse population. *Proc. R. Soc. B* 260, 215-256.

Keller, L.F. (1998). inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). *Evolution* 52, 240-250.

Keller, L.F., Jeffery, K.J., Arcese, P., Beaumont, M.A., Hochachka, W.M., Smith, J.N.M. & Bruford, M.W. (2001). Immigration and the ephemerality of a natural population bottleneck: evidence from molecular markers. *Proc. R. Soc. B* 268, 1387-1394.

Lenormand, T. (2002). Gene flow and the limits to natural selection. *Trends Ecol. Evol.* 17, 183-189.

Lenth, R.V. (2022). emmeans: estimated marginal means. R package v1.8.2.

Li, X.Y. & Kokko, H. (2019). Sex-biased dispersal: a review of the theory. *Biol. Rev.* 94, 721-736.

Marr, A.B., Keller, L.F. & Arcese, P. (2002). Heterosis and outbreeding depression in descendants of natural immigrants to an inbred population of song sparrows (*Melospiza melodia*). *Evolution* 56, 131-142.

Martinig, A.R., McAdam, A.G., Dantzer, B., Lane, J.E., Coltman, D.W. & Boutin, S. (2020). The new kid on the block: immigrant males win big whereas females pay fitness cost after dispersal. *Ecol. Let.* 23, 430–438.

McDonald, T. K. & Yeaman, S. (2018). Effect of migration and environmental heterogeneity on the maintenance of quantitative genetic variation; a simulation study. *J. Evol. Biol.* 31, 1386-1399.

Millon, A., Lambin, X., Devillard, S. & Schaub, M. (2019). Quantifying the contribution of immigration to population dynamics: a review of methods, evidence and perspectives in birds and mammals. *Biol. Rev.* 94, 2049-2067.

Mobley, K.B., Granroth-Wilding, H., Ellman, M., Vähä, J.-P., Aykanat, T., Johnston, S.E., *et al.* (2019). Home ground advantage: Local Atlantic salmon have higher reproductive fitness than dispersers in the wild. *Sci. Adv.* 5, eaav1112.

Nietlisbach, P., Keller, L.F., Camenisch, G., Guillaume, F., Arcese, P., Reid, J.M. & Postma, E. (2017). Pedigree-based inbreeding coefficient explains more variation in fitness than heterozygosity at 160 microsatellites in a wild bird population. *Proc. R. Soc. B* 284, 20162763.

Nosil, P., Vines, T.H. & Funk, D.J. (2005). Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution* 59, 705-719.

Peterson, D.A., Hilborn, R. & Hauser, L. (2014). Local adaptation limits lifetime reproductive success of dispersers in a wild salmon metapopulation. *Nat. Comms.* 5, 3696.

Porter, C.K. & Benkman, C.W. (2017). Assessing the potential contributions of reduced immigrant viability and fecundity to reproductive isolation. *Am. Nat.* 189, 580-591.

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reid, J.M., Arcese, P. & Keller, L.F. (2006). Intrinsic parent-offspring correlation in inbreeding level in a song sparrow (*Melospiza melodia*) population open to immigration. *Am. Nat.* 168, 1-13.

Reid, J.M. & Arcese, P. (2020). Recent immigrants alter the quantitative genetic architecture of paternity in song sparrows. *Evol. Let.* 4, 124-136.

Reid, J.M., Arcese, P., Nietlisbach, P., Wolak, M.E., Muff, S., Dickel, L. & Keller, L.F. (2021). Immigration counter-acts local micro-evolution of a major fitness component: migration-selection balance in free-living song sparrows. *Evol. Let.* 5, 48-60.

Reid, J.M., Keller, L.F., Marr, A.B., Nietlisbach, P., Sardell, R.J. & Arcese, P. (2014). Pedigree error due to extra-pair reproduction substantially biases estimates of inbreeding depression. *Evolution* 68, 802–815.

Reid, J.M., Nietlisbach, P., Wolak, M.E., Keller, L.F. & Arcese, P. (2019). Individuals' expected genetic contributions to future generations, reproductive value, and short-term metrics of fitness in free-living song sparrows (*Melospiza melodia*). *Evol. Let.* 3, 271-285.

Rhode, J.M & Cruzan, M.B. (2005). Contributions of heterosis and epistasis to hybrid fitness. *Am. Nat.* 166, E124-E139.

Richards, C.M. (2000). Inbreeding depression and genetic rescue in a plant metapopulation. *Am. Nat.* 155, 383-394.

Richardson, J.L., Urban, M.C., Bolnick, D.I. & Skelly, D.K. (2014). Microgeographic adaptation and the spatial scale of evolution. *Trends Ecol. Evol.* 29, 165-176.

Robertson, E.P., Fletcher Jr, R.J., Cattau, C.E., Udell, B.J., Reichert, B.E., Austin, J.D. & Valle, D. (2018). Isolating the roles of movement and reproduction on effective connectivity alters conservation priorities for an endangered bird. *Proc. Natl. Acad. Sci.* 115, 8591-8596.

Rundle, H.D. & Whitlock, M.C. (2001). A genetic interpretation of ecologically dependent isolation. *Evolution* 55, 198-201.

Saccheri, I.J. & Brakefield, P.M. (2002). Rapid spread of immigrant genomes into inbred populations. *Proc. R. Soc. B* 269, 1073-1078.

Sardell, R.J., Keller, L.F., Arcese, P., Bucher, T. & Reid, J.M. (2010). Comprehensive paternity assignment: genotype, spatial location and social status in song sparrows *Melospiza melodia*. *Mol. Ecol.* 19, 4352-4364.

Sella, G. & Barton, N.H. (2019). Thinking about the evolution of complex traits in the era of genome-wide association studies. *Annu. Rev. Genom. Hum. Genet.* 20, 461-493.

Spigler, R.B., Theodorou, K. & Chang, S.M. (2017). Inbreeding depression and drift load in small populations at demographic disequilibrium. *Evolution* 71, 81-94.

Svensson, O., Gräns, J., Celander, M.C., Havenhand, J., Leder, E.H., Lindström, K., *et al.* (2017). Immigrant reproductive dysfunction facilitates ecological speciation. *Evolution* 71, 2510-2521.

Tallmon, D.A., Luikart, G. & Waples, R.S. (2004). The alluring simplicity and complex reality of genetic rescue. *Trends Ecol. Evol.* 19, 489-496.

Vasudev, D. & Fletcher Jr, R.J. (2016). Mate choice interacts with movement limitations to influence effective dispersal. *Ecol. Model.* 327, 65-73.

Whiteley, A.R., Fitzpatrick, S.W., Funk, W.C. & Tallmon, D.A. (2015). Genetic rescue to the rescue. *Trends Ecol. Evol.* 30, 42-49.

Wilson, A.G. & Arcese, P. (2008). Influential factors for natal dispersal in an avian island metapopulation. *J. Av. Biol.* 39, 341-347.

Wolak, M.E. (2012). nadiv: an R package to create relatedness matrices for estimating non-additive genetic variances in animal models. *Methods Ecol. Evol.* 3, 792–796.

Wolak, M.E. & Reid, J.M. (2016). Is pairing with a relative heritable? Estimating female and male genetic contributions to the degree of biparental inbreeding in song sparrow (*Melozpiza melodia*). *Am. Nat.* 187, 736-752.

Zajitschek, S.R.K., Zajitschek, F. & Brooks, R.C. (2009). Demographic costs of inbreeding revealed by sex-specific genetic rescue effects. *BMC Evol. Biol.* 9, 289.

Zhang, L., Hood, G.R., Roush, A.M., Shzu, S.A., Comerford, M.S., Ott, J.R. & Egan, S.P. (2021). Asymmetric, but opposing reductions in immigrant viability and fecundity promote reproductive isolation among host-associated populations of an insect herbivore. *Evolution* 75, 476-489.

Figure 1. Numbers of new female immigrants (filled red points) and male immigrants (filled blue points) that arrived in the focal population each year during 1990-2012, and numbers of female natives (open red points) and male natives (open blue points) that recruited in the years when immigrants arrived. Solid versus dashed vertical lines highlight years in which at least one versus zero new immigrant arrived. The absolute number of new immigrants arriving per year did not change during 1990-2012 (Poisson regression slope β=-0.01, 95%CI -0.06 – 0.05, p=0.88 across 23 years including years with zero immigrants; and β=-0.001, 95%CI -0.05 – 0.05, p=0.98 across 13 years with ≥1 immigrant).

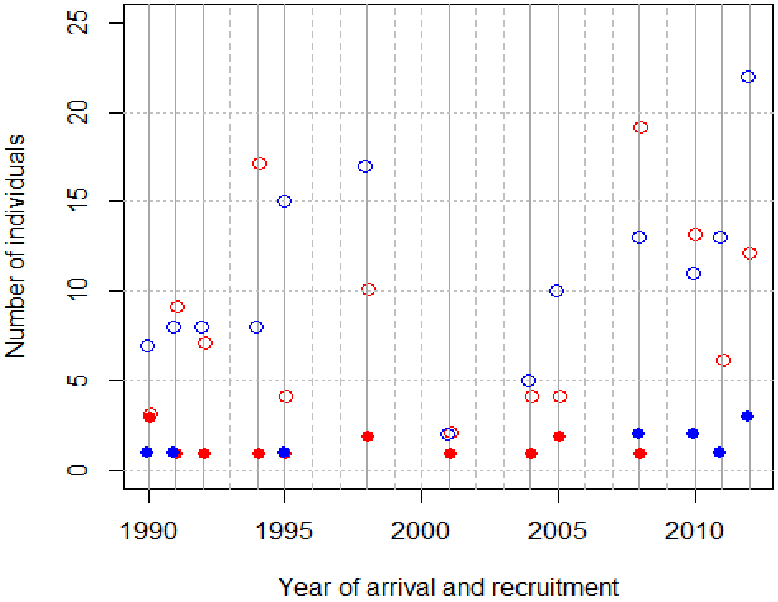


Figure 2. Genetic contributions made by all immigrants (filled grey points) and contemporary natives (open points) to the eight cohorts hatched following recruitment (years, where year 1 denotes the year of arrival for immigrants and the year after hatch for natives). Points show back-transformed weighted mean estimates across models fitted to N=1000 gene-drop iterations offset by cohort size, representing proportional realised genetic contributions (Prop$_{RGC}$). Whiskers show mean 95% confidence intervals. Black squares show weighted mean latent (log) scale effects of being an immigrant versus a native, with mean 95% confidence intervals. The dashed horizontal line denotes zero. Y-axis units are multiplied by 100, representing percentage contributions.

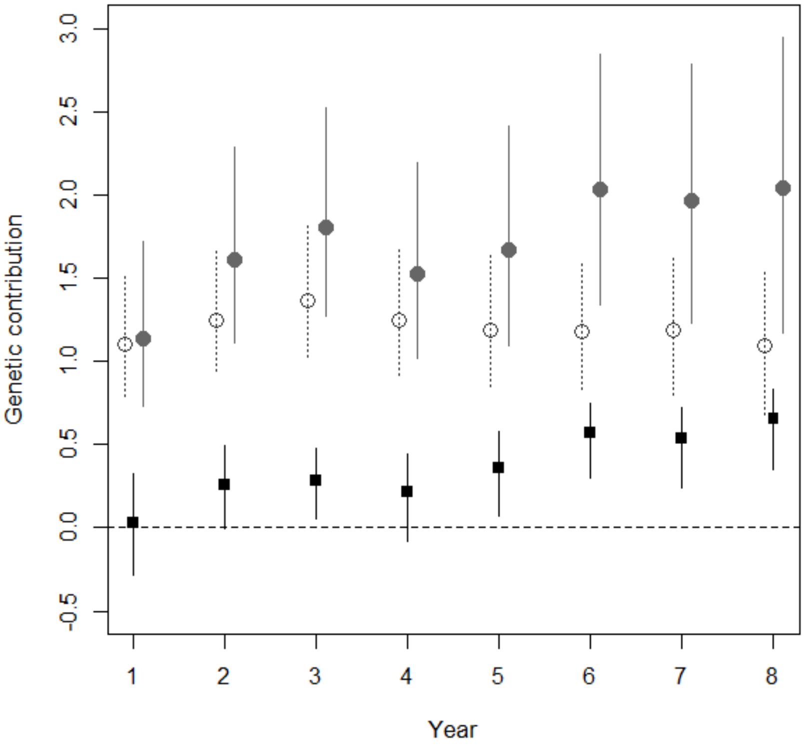


Figure 3. Genetic contributions made by (A) all females (red) and males (blue), (B) female immigrants (filled red) and natives (open red) and (C) male immigrants (filled blue) and natives (open blue) to cohorts hatched in the eight years following recruitment (x-axis), and (D) the status-by-sex interactions. Year 1 denotes the year of arrival for immigrants and the year after hatch for natives. Coloured points show back-transformed weighted mean estimates across models fitted to *N*=1000 gene-drop iterations offset by cohort size, representing proportional realised genetic contributions ($\mathrm{Prop}_{\mathrm{RGC}}$). Black points show weighted mean latent (log) scale estimates of the (A) male-female difference, (B,C) sex-specific immigrant-native differences and (D) status-by-sex interaction. Whiskers show mean 95% confidence intervals. Dashed horizontal lines denote zero. Y-axis units are multiplied by 100, representing percentage contributions (y-axis scales differ among panels).

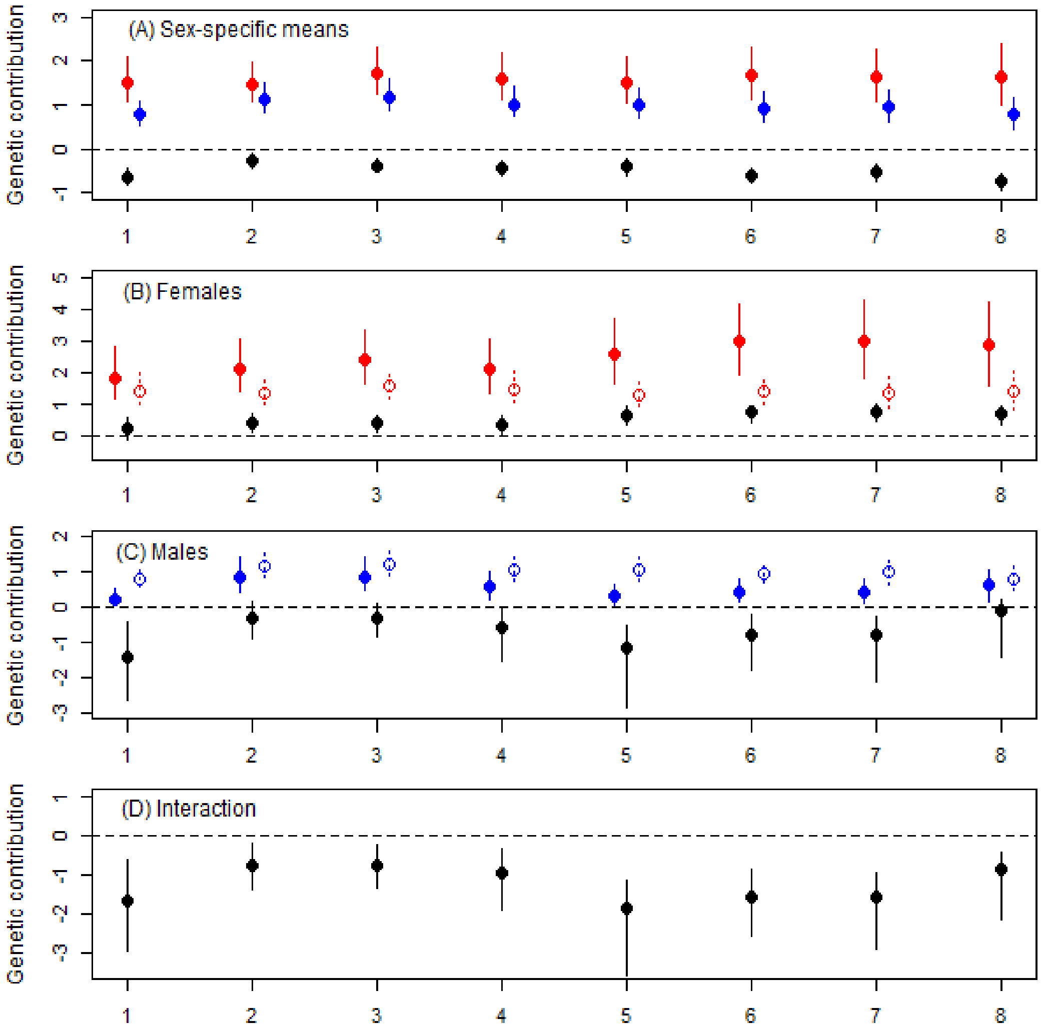

(A) Sex-specific means
(B) Females
(C) Males
(D) Interaction
Genetic contribution
-1
0
1
2
3
4
5
-3
-2
1
2
3
4
5
6
7
8

Figure 4. Frequency distributions of individuals' mean proportional genetic contributions ($\mu\mathrm{Prop}_{RGC}$) to the recipient population in the eighth year from recruitment in (A) females and (B) males, and (C) the relationship between $\mu\mathrm{Prop}_{RGC}$ and the probability that a unique allele of each focal individual will be locally extinct by year eight (P_0). Red and blue denote females and males respectively, and filled and open markers denote immigrants and natives respectively. On (A) and (B), y-axes are truncated; totals of 62 females and 90 males had zero $\mu\mathrm{Prop}_{RGC}$ respectively. On (C) these individuals are denoted by the larger circles for P_0 = 1. The black line depicts a log-linear regression fitted through all individuals (slope β=-0.25, 95%CI -0.27 – -0.23). $\mu\mathrm{Prop}_{RGC}$ values are multiplied by 100, representing percentage contributions.

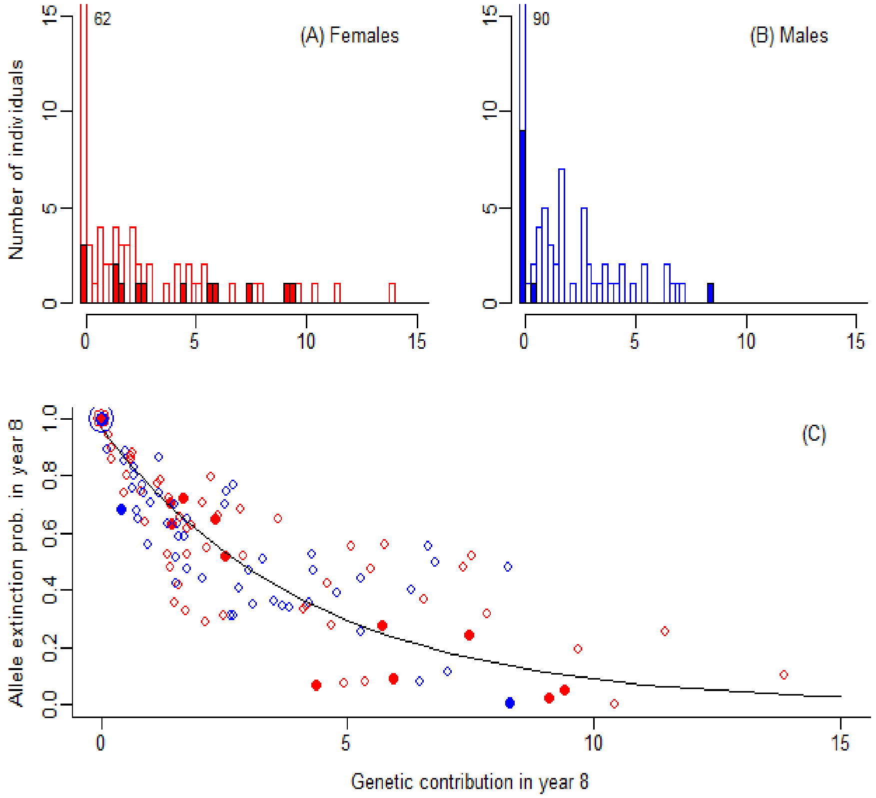


Figure 5. (A) Genetic contributions made by immigrant (filled red points) versus native (open red points) females to the cohorts of chicks hatched over fifteen years from recruitment (years, where year 1 is the year of arrival for immigrants and the year after hatch for natives), and (B) the relationship between genetic contribution and allele extinction probability (P_0) in year fifteen. On (A), red points show back-transformed weighted mean estimates across models fitted to N=1000 gene-drop iterations offset by cohort size, representing proportional genetic contributions (Prop$_{\text{RGC}}$). Black points show weighted mean latent (log) scale estimates of the immigrant-native difference. Whiskers show weighted mean 95% confidence intervals. The dashed horizontal line denotes zero. Estimates for years 1-8 differ slightly from those in Figure 3A, because one female immigrant that arrived in 2008 is excluded from the 15-year analysis and model structures differ. On (B), the black line denotes a fitted log-linear regression (slope β = -0.11, 95%CI -0.12 – -0.10). Totals of 31 natives and 3 immigrants had Prop$_{\text{EGC}}$ of zero (hence P_0=1), denoted by the larger point and circle. Genetic contribution values are multiplied by 100, representing percentage contributions.

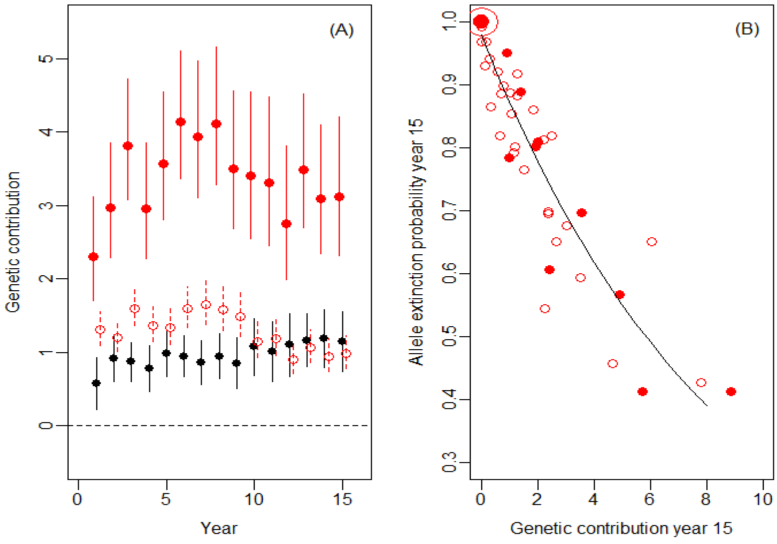
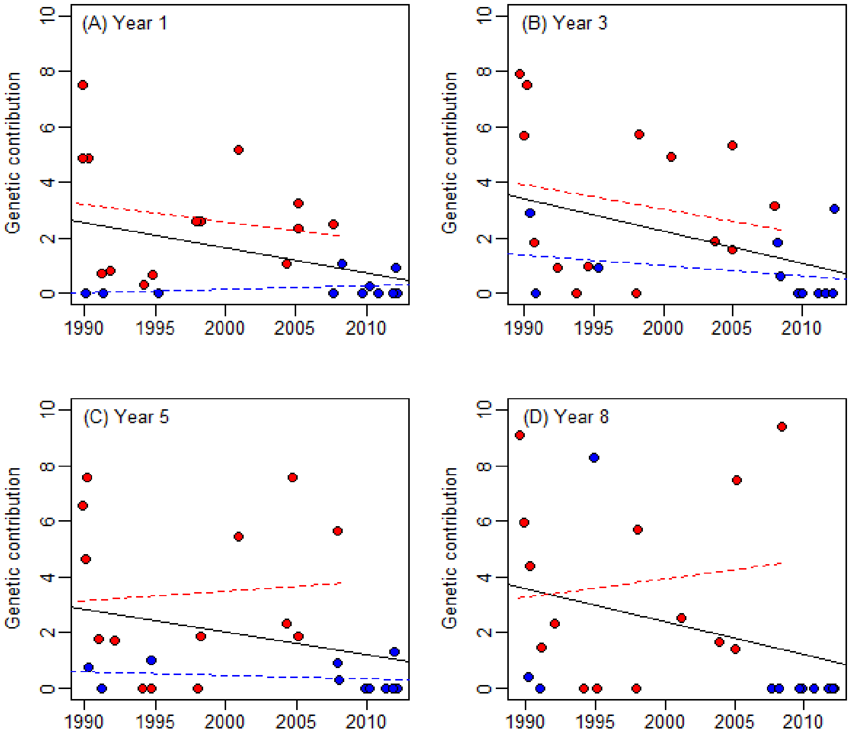


Figure 6. Relationships between mean proportional genetic contributions (μProp$_{\mathrm{RGC}}$) of new immigrants to the focal recipient population in four selected years from arrival: (A) one, (B) three, (C) five and (D) eight, and the calendar year of arrival (x-axis). Red and blue points denote female and male immigrants respectively. Red and blue dashed lines show respective sex-specific regressions of μProp$_{\mathrm{RGC}}$ on arrival year (not shown for males in panel D, because all males had values of or close to zero except one outlier whose μProp$_{\mathrm{RGC}}$ increased substantially between years 5 and 8). Slopes do not differ significantly from zero (all $p \geq 0.48$). Black solid lines show linear regressions across both sexes combined. Here, all slopes were negative (slopes [95% confidence intervals] are -0.09 [-0.19,0.01], -0.12 [-0.24,-0.001], -0.08 [-0.21, 0.04] and -0.12 [-0.28, 0.04] for A-D respectively). Genetic contribution values are multiplied by 100, representing percentage contributions.



Supporting Information for:

Multi-generation genetic contributions of immigrants reveal cryptic elevated and sex-biased effective gene flow within a natural meta-population

Jane M. Reid, Lisa Dickel, Lukas F. Keller, Pirmin Nietlisbach & Peter Arcese

Supporting Information S1: Definitions of abbreviations

Table S1. Definitions of key abbreviations for quantities calculated from pedigree data.

Abbreviation	**Definition**
$\mathbf{Tot_{EGC}}$	An individual's total expected genetic contribution (EGC) to a particular cohort of chicks, as can be directly calculated from complete pedigree data.
$\mathbf{Tot_{RGC}}$	An individual's total realised genetic contribution (RGC) to a particular cohort of chicks, computed from gene-drop simulations on pedigree data. 'Realised' refers to the outcome of a gene-drop of a hypothetical allele, not to any real-life outcome (i.e. genotype).
$\mathbf{\mu Tot_{RGC}}$	The mean of $\mathbf{Tot_{RGC}}$ calculated across N=1000 gene-drop

	simulations.
$\mathrm{Prop}_{\mathrm{RGC}}$	Predicted proportional **$\mathrm{Tot}_{\mathrm{RGC}}$**, accounting for the total number of chicks in each cohort as an offset variable.
$\mu\mathrm{Prop}_{\mathrm{RGC}}$	**$\mu\mathrm{Tot}_{\mathrm{RGC}}$** divided by the total number of chicks in each cohort, giving a proportional contribution.
P_0	Probability that a focal individual's (hypothetical) allele will be locally extinct at some specified number of years following the individual's recruitment.

Supporting Information S2: Details of pedigree analyses

Pedigrees were prepared, and genetic contributions (Table S1) estimated, by implementing the following steps for each focal cohort of song sparrows. A 'focal cohort' is defined as the cohort hatched the year before each focal immigrant arrived. This comprises all individuals that could be alive age one year when the immigrant arrived, which includes all defined contemporary 'natives'.

1. The full pedigree was cut to all individuals hatched in the focal cohort and all subsequent individuals, by deleting all individuals hatched before the focal cohort.
2. Both parents of all focal cohort individuals were set to NA, therefore effectively defining all focal cohort individuals as pedigree founders. This imposes the assumption that all focal cohort individuals are unrelated to each other. While this is clearly not realistic, it allows easy calculation of

genetic contributions arising through direct descendants of each focal individual but not through non-lineal relatives (e.g. nieces and nephews, which are offspring of siblings). This is as required for current purposes.

3. Immigrants that arrived in the year following the focal cohort's natal year were added as further pedigree founders, with parents set as NA.
4. All remaining individuals in the pedigree whose parents had been cut were assigned dummy founder parents. These comprised individuals hatched after the focal cohort whose real parents hatched previously, or were future immigrants that arrived >1 year after the focal cohort hatched.
5. All founders (i.e. with NA parents) were assigned a unique numeric allele value, with their maternal and paternal alleles denoted by + and -.
6. These hypothetical alleles were then 'dropped' down the prepared pedigree with autosomal Mendelian inheritance (i.e., transmitted to each offspring of each sex with probability 0.5), as implemented in the 'geneDrop' function in R package nadiv (Wolak 2012). The resulting dataframe comprises the two alleles inherited by each individual in the pedigree.
7. All chicks hatched in each year following the focal cohort's natal year were identified. The total number of copies of each unique allele of each focal cohort individual that was present in each set of identified chicks was computed. This gives each focal individual's total realised genetic contribution (Tot_{RGC}) to the cohort of chicks hatched in each subsequent year, for one gene drop iteration.

8. Steps 6 and 7 were repeated for N=1000 iterations, giving 1000 values of $\mathrm{Tot}_{\mathrm{RGC}}$ for each focal individual. The probability that a focal individual's allele would go extinct (P_0) was then computed as the proportion of the N=1000 gene drops where $\mathrm{Tot}_{\mathrm{RGC}}=0$. An individual's mean realised genetic contribution ($\mu\mathrm{Tot}_{\mathrm{RGC}}$) to each cohort was additionally estimated as the mean across its 1000 values of $\mathrm{Tot}_{\mathrm{RGC}}$.

9. Steps 1-8 were repeated for each focal cohort (i.e. the 13 cohorts that hatched in the years before the focal immigrants arrived, see main Figure 1).

Points to note:

- Gene drop simulations yield stochastic outcomes for $\mathrm{Tot}_{\mathrm{RGC}}$ due to the probabilistic Mendelian inheritance of alleles across generations (a simple example is shown in Figure S1). This in turn yields an intrinsic mean-variance relationship. For individuals with few or no pedigree descendants and hence small $\mathrm{Tot}_{\mathrm{EGC}}$, $\mathrm{Tot}_{\mathrm{RGC}}$ will always be small or zero. For individuals with several pedigree descendants and hence larger $\mathrm{Tot}_{\mathrm{EGC}}$, $\mathrm{Tot}_{\mathrm{RGC}}$ for any gene drop iteration could be small or zero, or could be larger, depending on how many allele copies are retained through the stochastic inheritance process (Figure S1).
- The gene drop algorithm encompasses any inbreeding among any focal individual's descendants. Hence, one descendant can in principle inherit two copies of a focal individual's maternal or paternal allele.

- $\mathrm{Tot}_{\mathrm{RGC}}$ was computed across the chicks hatched in each year, not across all individuals alive in each year (including adults). This means that $\mathrm{Tot}_{\mathrm{RGC}}$ for a focal individual in a particular year can temporarily be zero, if it and/or its descendant(s) were still alive but failed to reproduce in a particular year then did so subsequently. However, there were never more than 1 or 2 years of $\mathrm{Tot}_{\mathrm{RGC}}=0$ followed by a non-zero value for any individual. The lineages of all individuals with $\mathrm{Tot}_{\mathrm{RGC}}=0$ in year 8 (i.e. seven years after their focal cohort recruited) had truly gone extinct and did not reappear subsequently.

- The variance in $\mathrm{Tot}_{\mathrm{RGC}}$ and in P_0 given $\mu\mathrm{Tot}_{\mathrm{RGC}}$ reflects the pedigree structures of individuals' descendants. For example, individuals that had few offspring that then bred successfully could have the same medium-term $\mu\mathrm{Tot}_{\mathrm{RGC}}$ as individuals that had more offspring that then bred less successfully, but these individuals could differ in the variance in $\mathrm{Tot}_{\mathrm{RGC}}$ and in P_0.

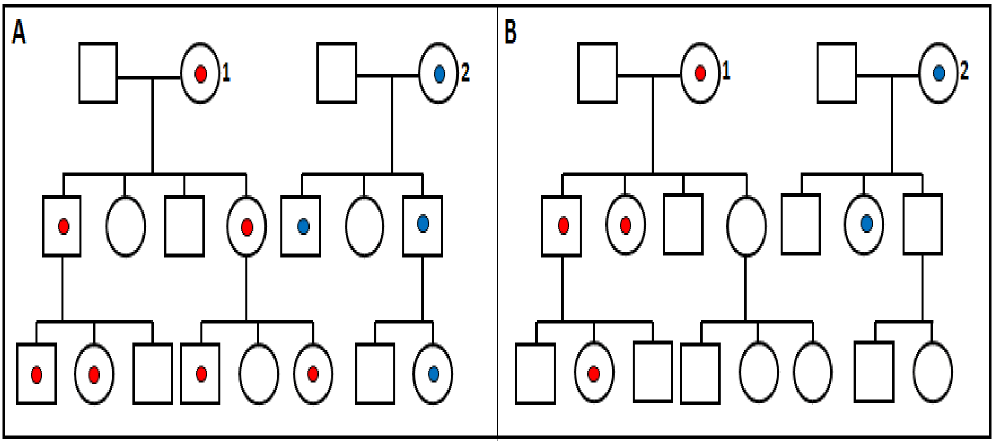
- The prepared pedigrees, and hence gene drop runs, include F1, F2 and backcross descendants of previous immigrants as founders. However these individuals were excluded from subsequent analyses, which were restricted to immigrants and defined natives (i.e. locally-hatched individuals with four locally-hatched grandparents).

- There were no instances where two immigrants mated with each other.

- To further verify estimates of μTot_{RGC} from the gene drops and hence check for errors, we additionally directly computed each focal individual's coefficient of kinship with all other individuals in the pedigree. This was implemented by assigning each focal individual to its own 'genetic group', then using the 'ggcontrib' function in R package nadiv (Wolak 2012; Wolak & Reid 2017). These coefficients of kinship were then summed across all chicks hatched in each subsequent year to give each focal individual's Tot_{EGC}. Direct statistical analyses of Tot_{EGC} yielded very similar results as the analyses of Tot_{RGC} presented in the main text (i.e. evidence of elevated and female-biased contributions of immigrants).

- All gene drop simulations involve hypothetical alleles, not particular microsatellite alleles, or any other real genetic markers that have been evaluated in our focal pedigreed individuals. Rather, our microsatellite data were solely used to assign paternity and hence construct the pedigree on which the gene drop was implemented.

Figure S1. Illustration of a simple pedigree and gene drop simulation. Squares and circles denote males and females respectively, showing two focal pairings (top row), and their offspring (middle row) and grandoffspring (bottom row). For simplicity, mates of breeding offspring are not shown. Hypothetical alleles (red and blue points) initiated in the two focal females are 'dropped' down the pedigree assuming autosomal Mendelian inheritance (i.e. probability of 0.5 of transmission to each offspring). In the illustrated example, female 1 (red allele) represents a new immigrant and female 2 (blue allele) represents a contemporary native. Panels A and B depict two plausible outcomes of the gene drop on the exact same pedigree, showing that the red and blue alleles are stochastically inherited by different sets of offspring and grandoffspring. Values of Tot$_{RGC}$ for females 1 and 2 in the grandoffspring generation are 4 and 1 respectively in panel A, and 1 and 0 respectively in panel B.



Supporting Information S3: Details of statistical analyses

In total, we fitted 8000 models for the short-term analyses of Tot_{RGC} in both sexes, and 15000 models for the longer-term analyses for females only (i.e. 1000 x 8 and 15 years respectively). This required handling a small number of models that did not converge.

For the short-term analyses of both sexes, models did not always converge for gene-drop iterations where Tot_{RGC} was zero for all 11 immigrant males. This occurred in 13, 0, 0, 0, 129, 0, 73 and 6 of 1000 iterations in years one to eight from immigrants' arrivals respectively. The high total in year five occurred because the one ultimately successful male immigrant had low Tot_{EGC}, which subsequently increased due to success of the male's descendants (see main Figures 6C-D). This was resolved by increasing the successful male immigrant's value of Tot_{RGC} from zero to one in cases where all 11 male immigrants' values were zero. This small numerical increase facilitated model convergence. It means that the difference in Prop_{RGC} between immigrant and native males, and the sex by status interaction, will be slightly underestimated (i.e. conservative) in year five (and negligibly so otherwise). There were no gene-drop iterations where Tot_{RGC} was zero for all 14 immigrant females in any of the first eight years from arrival.

However, for the longer-term analyses of females only, Tot_{RGC} was zero for all 13 considered immigrant females in 1, 5, 8, 13, 13 and 22 of 1000 gene drop iterations in years 10-15 from arrival respectively, again impeding model convergence. This was again resolved by increasing the most successful female immigrant's value of $\mathrm{Tot}_{\mathrm{RGC}}$ from zero to one in cases where all 13 female immigrants' values were zero. This small numerical increase again facilitated model convergence but had negligible quantitative effects on results.

Since there were few (≤3) immigrants per year and immigrants' sexes were unequally distributed across years (main Figure 1), we did not attempt to estimate sex or status by recruitment year interactions.

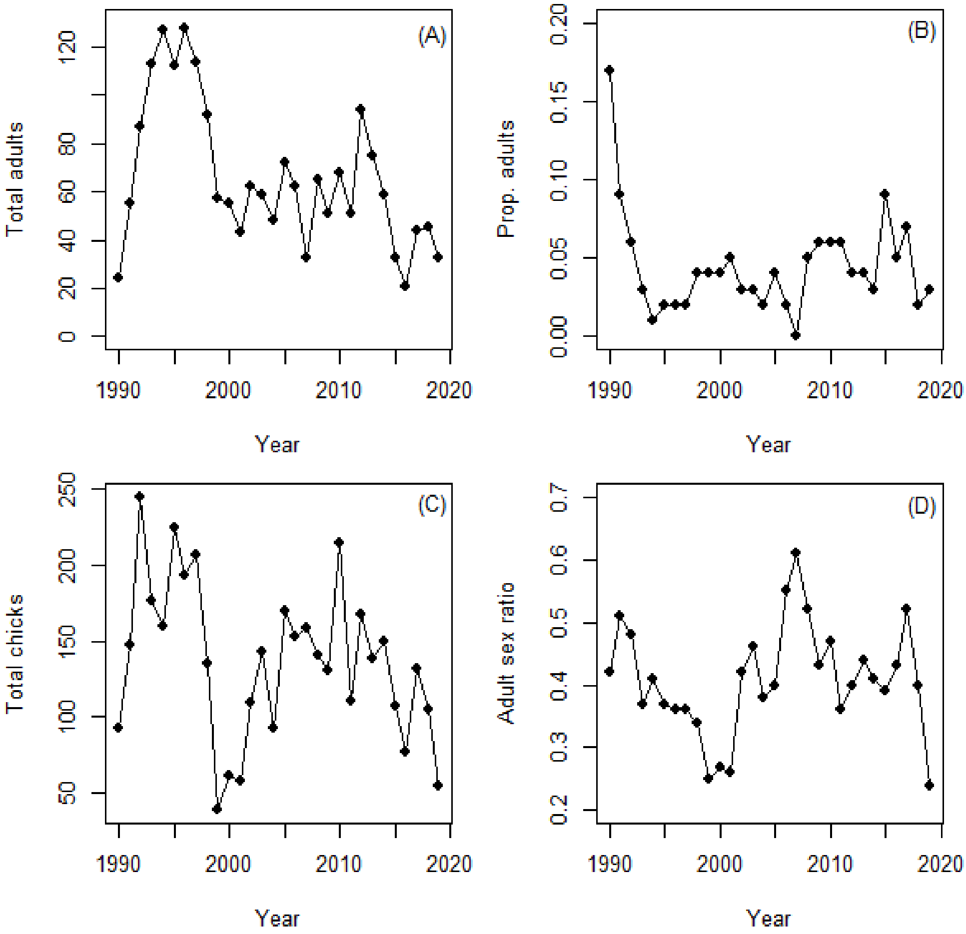
The overall higher $\mathrm{Prop}_{\mathrm{RGC}}$ for females versus males reflects two effects. First, the local adult sex ratio is commonly male-biased (Supporting Information S4), meaning that not all surviving adult males breed in all years. Second, mating and reproductive success of male F1 offspring of immigrants is relatively high (consistent with heterosis, Dickel et al. 2024, Supporting Information S6). Since F1 individuals are not included as focal natives in current analyses, some relatively successful males were therefore excluded, decreasing the sex-specific mean of the focal males.

Supporting Information S4: Details of population size and composition

Figure S1 shows the total number of locally-hatched adult song sparrows alive on Mandarte during 1990-2019, the total proportion of adults that were immigrants (either newly arrived or surviving from previous years) in each year, the total numbers of chicks ringed in each year, and the overall adult sex ratio (proportion female). Descriptive statistics (mean±SD and range) are: total adults: 73±30 (24-128); proportion immigrants: 0.04±0.03 (0.00-0.17); total chicks: 136±52 (38-245); adult sex ratio: 0.41±0.09 (0.24-0.61).

The proportion of new recruits that were immigrants did not change during the years when the focal immigrants arrived (i.e. 1990-2012, binomial regression slope $\beta=-0.01$, 95%CI -0.06 – 0.05, $p=0.88$ across 23 years including years with zero immigrants; and $\beta=-0.001$, 95%CI -0.05 – 0.05, $p=0.98$ across 13 years with ≥1 immigrant). The high proportion of immigrants in 1990 followed a documented local population crash in 1989 (Keller et al. 2001). Due to the immigration, the mean population-wide coefficient of inbreeding (f) increased only slightly during 1990-2019, from ca. 0.06 to 0.09 (Reid et al. 2021).

Figure S2. (A) Total numbers of locally hatched adult song sparrows alive on Mandarte, (B) proportions of the total adults that were immigrants, (C) total numbers of chicks ringed, and (D) overall adult sex ratio (proportion female) in each year during 1990-2019.



Supporting Information S5: Summaries of statistical tests and effect sizes

Allele extinction probabilities by year eight

Local allele extinction probabilities by year eight from recruitment were higher for immigrant males than immigrant females (male immigrants: 0.88, median 1.00, range 0.01-1.00; female immigrants: mean 0.50, median 0.58, range 0.02-1.00; *t*=2.8, bootstrap p≈0.03). They were also higher for native females than immigrant females (female natives: mean 0.80, median 1.00, range 0.00-1.00; *t*=2.9, bootstrap p≈0.01), but did not differ between native and immigrant males (male natives: mean 0.84, median 1.00, range 0.08-1.00; *t*=0.4, bootstrap p≈0.78).

Allele extinction probabilities by year fifteen

Local allele extinction probabilities by year fifteen from recruitment were higher for native females than immigrant females (natives: 0.90, median 1.00, range 0.43-1.00; immigrants: mean 0.76, median 0.80, range 0.41-1.00; *t*=2.2, bootstrap p≈0.03).

Data plotted in figures 2, 3 and 5A are provided in tables S2-S4 below.

Abbreviations: i=immigrant, n=native, f=female, m=male, status=immigrant versus native, sex=male versus female, interac=status by sex interaction, eff=weighted mean effect, lci=lower confidence interval, uci=upper confidence interval. Effects and confidence intervals for female and male immigrants and natives are back-transformed onto the observed scale. Effects and confidence intervals for the status, sex and interaction effects are on the modelled latent (log) scale. Rows denote years 1-8 for short-term analyses and years 1-15 for longer-term analyses.

Table S2. Data for figure 2.

n.eff	**n.lci**	**n.uci**	**i.eff**	**i.lci**	**i.uci**	**status.eff**	**status.lci**	**status.uci**
1.10	0.79	1.53	1.13	0.73	1.72	0.03	-0.29	0.32
1.25	0.94	1.66	1.61	1.11	2.28	0.26	-0.01	0.50
1.37	1.03	1.83	1.81	1.27	2.53	0.28	0.05	0.48
1.25	0.92	1.70	1.53	1.02	2.20	0.21	-0.08	0.44
1.19	0.85	1.63	1.67	1.10	2.41	0.36	0.07	0.58
1.18	0.83	1.60	2.03	1.34	2.84	0.57	0.30	0.75
1.19	0.80	1.62	1.97	1.23	2.78	0.53	0.24	0.72
1.09	0.68	1.55	2.04	1.18	2.95	0.66	0.35	0.84

Table S3A. Data for figure 3A.

f.eff	**f.lci**	**f.uci**	**m.eff**	**m.lci**	**m.uci**	**sex.eff**	**sex.lci**	**sex.uci**
1.50	1.07	2.09	0.78	0.55	1.10	-0.65	-0.84	-0.46
1.47	1.09	1.99	1.13	0.83	1.52	-0.27	-0.45	-0.10
1.71	1.26	2.31	1.17	0.86	1.59	-0.38	-0.53	-0.22
1.59	1.14	2.19	1.02	0.73	1.41	-0.44	-0.63	-0.27
1.51	1.06	2.12	0.99	0.69	1.40	-0.41	-0.60	-0.24
1.67	1.15	2.32	0.92	0.62	1.28	-0.59	-0.78	-0.43
1.64	1.08	2.29	0.94	0.62	1.33	-0.54	-0.73	-0.37
1.65	1.00	2.42	0.79	0.47	1.16	-0.73	-0.94	-0.55

Table S3B. Data for figure 3B.

f.n.eff	**f.n.lci**	**f.n.uci**	**f.i.eff**	**f.i.lci**	**f.i.uci**	**status.eff**	**status.lci**	**status.uci**
1.43	1.02	1.99	1.85	1.19	2.83	0.26	-0.10	0.59
1.36	1.00	1.82	2.12	1.43	3.05	0.45	0.13	0.74
1.57	1.17	2.11	2.39	1.65	3.36	0.42	0.15	0.67
1.48	1.07	2.03	2.13	1.38	3.08	0.37	0.03	0.64

1.32	0.93	1.82	2.58	1.67	3.70	0.68	0.36	0.94
1.42	0.99	1.94	2.98	1.93	4.17	0.75	0.46	0.98
1.39	0.91	1.91	3.01	1.84	4.26	0.79	0.47	1.03
1.41	0.86	2.05	2.87	1.61	4.21	0.73	0.38	0.96

Table S3C. Data for figure 3C.

m.n.eff	**m.n.lci**	**m.n.uci**	**m.i.eff**	**m.i.lci**	**m.i.uci**	**status.eff**	**status.lci**	**status.uci**
0.83	0.59	1.16	0.20	0.06	0.56	-1.40	-2.67	-0.43
1.15	0.86	1.54	0.83	0.45	1.45	-0.32	-0.88	0.18
1.21	0.90	1.62	0.86	0.48	1.42	-0.33	-0.85	0.10
1.06	0.76	1.46	0.58	0.22	1.04	-0.59	-1.51	-0.07
1.06	0.75	1.47	0.32	0.06	0.65	-1.17	-2.86	-0.52
0.98	0.68	1.35	0.42	0.15	0.82	-0.81	-1.79	-0.20
1.00	0.66	1.39	0.43	0.11	0.79	-0.78	-2.12	-0.25
0.83	0.50	1.20	0.67	0.17	1.04	-0.09	-1.42	0.21

Table S3D. Data for figure 3D.

interac.eff	**interac.lci**	**interac.uci**
-1.66	-2.97	-0.62
-0.77	-1.40	-0.17
-0.75	-1.33	-0.24
-0.95	-1.92	-0.34
-1.85	-3.55	-1.13
-1.55	-2.55	-0.88
-1.57	-2.92	-0.96
-0.83	-2.16	-0.40

Table 4. Data for figure 5A.

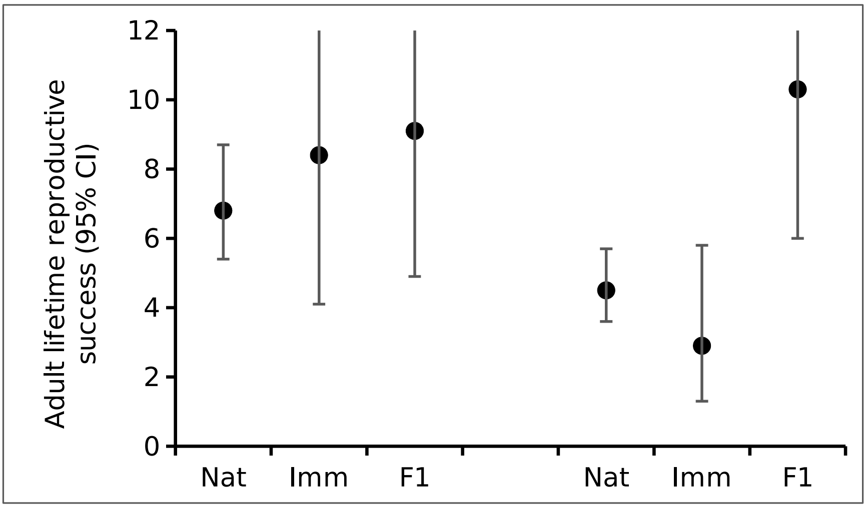
f.i.eff	**f.i.lci**	**f.i.uci**	**f.n.eff**	**f.n.lci**	**f.n.uci**	**status.eff**	**status.lci**	**status.uci**
2.30	1.70	3.12	1.30	1.09	1.55	0.57	0.22	0.92
2.97	2.30	3.85	1.20	1.00	1.43	0.91	0.59	1.22
3.82	3.08	4.73	1.59	1.36	1.86	0.87	0.60	1.14
2.96	2.28	3.85	1.36	1.15	1.62	0.78	0.46	1.09
3.58	2.80	4.56	1.33	1.11	1.59	0.98	0.67	1.29
4.14	3.36	5.10	1.59	1.33	1.89	0.94	0.67	1.22
3.93	3.11	4.98	1.65	1.38	1.98	0.86	0.56	1.16
4.12	3.28	5.16	1.58	1.30	1.91	0.94	0.64	1.25
3.50	2.69	4.56	1.48	1.21	1.81	0.85	0.50	1.19
3.40	2.54	4.55	1.15	0.92	1.43	1.07	0.68	1.46

3.32	2.45	4.49	1.19	0.95	1.50	1.01	0.60	1.42
2.75	1.99	3.81	0.90	0.71	1.14	1.10	0.67	1.53
3.49	2.69	4.53	1.06	0.86	1.31	1.16	0.80	1.52
3.10	2.34	4.10	0.94	0.74	1.18	1.18	0.79	1.58
3.12	2.31	4.21	0.98	0.78	1.22	1.15	0.74	1.55

Supporting Information S6: Heterosis in offspring of immigrants

Previous analyses using our song sparrow dataset showed evidence of strong heterosis, manifested as increased fitness in F1 offspring of immigrant-native pairings (Dickel et al. 2024). Specifically, male F1s have much higher adult lifetime reproductive success (LRS, defined as the total number of hatched offspring) than natives or immigrants, with much weaker effects in female F1s (Figure S3). Meanwhile, LRS tended to be higher in female immigrants than female natives, but lower in male immigrants than male natives, but these effects are not strongly statistically supported (Figure S3). These results partly reflect that male F1s are more likely to acquire a socially-paired female than other males, while male immigrants are less likely to mate successfully. These outcomes in turn partly reflect strong inbreeding depression in male song repertoire size (a secondary sexual trait, Reid et al. 2005), leading to low initial pairing success (Reid et al. 2004), and very strong inbreeding depression in male success in extra-pair reproduction (Reid et al. 2011).

Figure S3. Summary of key results from Dickel et al. (2024), showing the posterior mean (and 95% credible interval) adult lifetime reproductive success (total chicks hatched) for native (Nat), immigrant (Imm) individuals and their F1 offspring, for females (red) and males (blue). F1 offspring are modelled according to their own sex, not the sex of their immigrant parent. Full details of these analyses, and similar analyses for other fitness components, are in Dickel et al. (2024).



References

Dickel, L., Arcese, P., Keller, L.F., Nietlisbach, P., Goedert, D., Jensen, H. & Reid, J.M. (2024). Multi-generational fitness effects of natural immigration indicate strong heterosis and epistatic breakdown in a wild bird population. *Am. Nat.*, in press.

Keller, L.F., Jeffery, K.J., Arcese, P., Beaumont, M.A., Hochachka, W.M., Smith, J.N.M. & Bruford, M.W. (2001). Immigration and the ephemerality of a natural population bottleneck: evidence from molecular markers. *Proc. R. Soc. B* 268, 1387-1394.

Reid, J.M., Arcese, P., Nietlisbach, P., Wolak, M.E., Muff, S., Dickel, L. & Keller, L.F. (2021). Immigration counter-acts local micro-evolution of a major fitness component: migration-selection balance in free-living song sparrows. *Evol. Let.* 5, 48-60.

Reid, J.M., Arcese, P., Cassidy, A.L.E.V., Marr, A.B., Smith, J.N.M. & Keller, L.F. (2005). Song repertoire size indicates inbreeding and immunity in song sparrows. *Proc. R Soc. B* 272, 481-487.

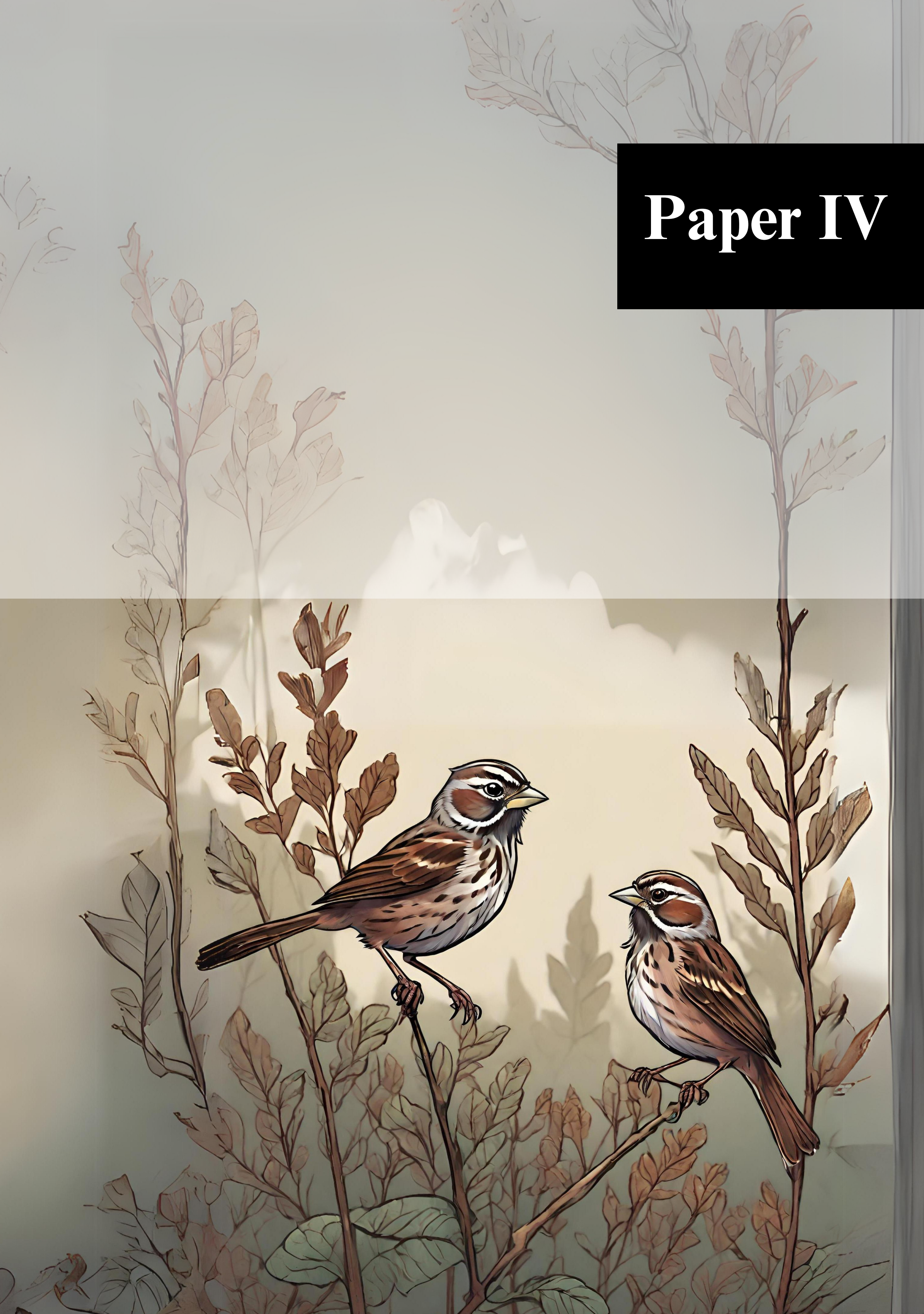
Reid, J.M., Arcese, P., Cassidy, A.L.E.V., Hiebert, S.M., Marr, A.B., Smith, J.N.M., Stoddard, P.K. & Keller, L.F. (2004). Song repertoire size predicts initial mating success. *Anim. Behav.* 68, 1055-1063.

Reid, J.M., Arcese, P., Sardell, R.J. & Keller, L.F. (2011). Additive genetic variance, heritability and inbreeding depression in male extra-pair reproductive success. *Am. Nat.* 177, 177-187.

Wolak, M.E. (2012). nadiv: an R package to create relatedness matrices for estimating non-additive genetic variances in animal models. *Methods Ecol. Evol.* 3, 792–796.

Wolak, M.E. & Reid, J.M. (2017). Accounting for genetic differences among unknown parents in micro-evolutionary studies: how to include genetic groups in quantitative genetic animal models. *J. Anim. Ecol.* 86, 7-20.

Paper IV



Dickel, Lisa; Goedert, Debora; Keller, Lukas F.; Arcese, Peter; Reid, Jane Margaret.
Does non-random mating modulate the multi-generational fitness effects of immigration?

This paper will be submitted for publication and is therefore not included.

Doctoral theses in Biology
Norwegian University of Science and Technology
Department of Biology

Year	Name	Degree	Title
1974	Tor-Henning Iversen	Dr. philos Botany	The roles of statholiths, auxin transport, and auxin metabolism in root gravitropism
1978	Tore Slagsvold	Dr. philos Zoology	Breeding events of birds in relation to spring temperature and environmental phenology
1978	Egil Sakshaug	Dr. philos Botany	The influence of environmental factors on the chemical composition of cultivated and natural populations of marine phytoplankton
1980	Arnfinn Langeland	Dr. philos Zoology	Interaction between fish and zooplankton populations and their effects on the material utilization in a freshwater lake
1980	Helge Reinertsen	Dr. philos Botany	The effect of lake fertilization on the dynamics and stability of a limnetic ecosystem with special reference to the phytoplankton
1982	Gunn Mari Olsen	Dr. scient Botany	Gravitropism in roots of *Pisum sativum* and *Arabidopsis thaliana*
1982	Dag Dolmen	Dr. philos Zoology	Life aspects of two sympartic species of newts (*Triturus, Amphibia*) in Norway, with special emphasis on their ecological niche segregation
1984	Eivin Røskaft	Dr. philos Zoology	Sociobiological studies of the rook *Corvus frugilegus*
1984	Anne Margrethe Cameron	Dr. scient Botany	Effects of alcohol inhalation on levels of circulating testosterone, follicle stimulating hormone and luteinzing hormone in male mature rats
1984	Asbjørn Magne Nilsen	Dr. scient Botany	Alveolar macrophages from expectorates – Biological monitoring of workers exposed to occupational air pollution. An evaluation of the AM-test
1985	Jarle Mork	Dr. philos Zoology	Biochemical genetic studies in fish
1985	John Solem	Dr. philos Zoology	Taxonomy, distribution and ecology of caddisflies (*Trichoptera*) in the Dovrefjell mountains
1985	Randi E. Reinertsen	Dr. philos Zoology	Energy strategies in the cold: Metabolic and thermoregulatory adaptations in small northern birds
1986	Bernt-Erik Sæther	Dr. philos Zoology	Ecological and evolutionary basis for variation in reproductive traits of some vertebrates: A comparative approach
1986	Torleif Holthe	Dr. philos Zoology	Evolution, systematics, nomenclature, and zoogeography in the polychaete orders *Oweniimorpha* and *Terebellomorpha*, with special reference to the Arctic and Scandinavian fauna
1987	Helene Lampe	Dr. scient Zoology	The function of bird song in mate attraction and territorial defence, and the importance of song repertoires
1987	Olav Hogstad	Dr. philos Zoology	Winter survival strategies of the Willow tit *Parus montanus*
1987	Jarle Inge Holten	Dr. philos Botany	Autecological investigations along a coust-inland transect at Nord-Møre, Central Norway

1987	Rita Kumar	Dr. scient Botany	Somaclonal variation in plants regenerated from cell cultures of *Nicotiana sanderae* and *Chrysanthemum morifolium*
1987	Bjørn Åge Tømmerås	Dr. scient Zoology	Olfaction in bark beetle communities: Interspecific interactions in regulation of colonization density, predator - prey relationship and host attraction
1988	Hans Christian Pedersen	Dr. philos Zoology	Reproductive behaviour in willow ptarmigan with special emphasis on territoriality and parental care
1988	Tor G. Heggberget	Dr. philos Zoology	Reproduction in Atlantic Salmon (*Salmo salar*): Aspects of spawning, incubation, early life history and population structure
1988	Marianne V. Nielsen	Dr. scient Zoology	The effects of selected environmental factors on carbon allocation/growth of larval and juvenile mussels (*Mytilus edulis*)
1988	Ole Kristian Berg	Dr. scient Zoology	The formation of landlocked Atlantic salmon (*Salmo salar* L.)
1989	John W. Jensen	Dr. philos Zoology	Crustacean plankton and fish during the first decade of the manmade Nesjø reservoir, with special emphasis on the effects of gill nets and salmonid growth
1989	Helga J. Vivås	Dr. scient Zoology	Theoretical models of activity pattern and optimal foraging: Predictions for the Moose *Alces alces*
1989	Reidar Andersen	Dr. scient Zoology	Interactions between a generalist herbivore, the moose *Alces alces*, and its winter food resources: a study of behavioural variation
1989	Kurt Ingar Draget	Dr. scient Botany	Alginate gel media for plant tissue culture
1990	Bengt Finstad	Dr. scient Zoology	Osmotic and ionic regulation in Atlantic salmon, rainbow trout and Arctic charr: Effect of temperature, salinity and season
1990	Hege Johannesen	Dr. scient Zoology	Respiration and temperature regulation in birds with special emphasis on the oxygen extraction by the lung
1990	Åse Krøkje	Dr. scient Botany	The mutagenic load from air pollution at two work-places with PAH-exposure measured with Ames Salmonella/microsome test
1990	Arne Johan Jensen	Dr. philos Zoology	Effects of water temperature on early life history, juvenile growth and prespawning migrations of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*): A summary of studies in Norwegian streams
1990	Tor Jørgen Almaas	Dr. scient Zoology	Pheromone reception in moths: Response characteristics of olfactory receptor neurons to intra- and interspecific chemical cues
1990	Magne Husby	Dr. scient Zoology	Breeding strategies in birds: Experiments with the Magpie *Pica pica*
1991	Tor Kvam	Dr. scient Zoology	Population biology of the European lynx (*Lynx lynx*) in Norway
1991	Jan Henning L'Abêe Lund	Dr. philos Zoology	Reproductive biology in freshwater fish, brown trout *Salmo trutta* and roach *Rutilus rutilus* in particular
1991	Asbjørn Moen	Dr. philos Botany	The plant cover of the boreal uplands of Central Norway. I. Vegetation ecology of Sølendet nature reserve; haymaking fens and birch woodlands
1991	Else Marie Løbersli	Dr. scient Botany	Soil acidification and metal uptake in plants
1991	Trond Nordtug	Dr. scient Zoology	Reflectometric studies of photomechanical adaptation in superposition eyes of arthropods
1991	Thyra Solem	Dr. scient Botany	Age, origin and development of blanket mires in Central Norway

1991	Odd Terje Sandlund	Dr. philos Zoology	The dynamics of habitat use in the salmonid genera *Coregonus* and *Salvelinus*: Ontogenic niche shifts and polymorphism
1991	Nina Jonsson	Dr. philos Zoology	Aspects of migration and spawning in salmonids
1991	Atle Bones	Dr. scient Botany	Compartmentation and molecular properties of thioglucoside glucohydrolase (myrosinase)
1992	Torgrim Breiehagen	Dr. scient Zoology	Mating behaviour and evolutionary aspects of the breeding system of two bird species: the Temminck's stint and the Pied flycatcher
1992	Anne Kjersti Bakken	Dr. scient Botany	The influence of photoperiod on nitrate assimilation and nitrogen status in timothy (*Phleum pratense* L.)
1992	Tycho Anker-Nilssen	Dr. scient Zoology	Food supply as a determinant of reproduction and population development in Norwegian Puffins *Fratercula arctica*
1992	Bjørn Munro Jenssen	Dr. philos Zoology	Thermoregulation in aquatic birds in air and water: With special emphasis on the effects of crude oil, chemically treated oil and cleaning on the thermal balance of ducks
1992	Arne Vollan Aarset	Dr. philos Zoology	The ecophysiology of under-ice fauna: Osmotic regulation, low temperature tolerance and metabolism in polar crustaceans.
1993	Geir Slupphaug	Dr. scient Botany	Regulation and expression of uracil-DNA glycosylase and O^6-methylguanine-DNA methyltransferase in mammalian cells
1993	Tor Fredrik Næsje	Dr. scient Zoology	Habitat shifts in coregonids.
1993	Yngvar Asbjørn Olsen	Dr. scient Zoology	Cortisol dynamics in Atlantic salmon, *Salmo salar* L.: Basal and stressor-induced variations in plasma levels and some secondary effects.
1993	Bård Pedersen	Dr. scient Botany	Theoretical studies of life history evolution in modular and clonal organisms
1993	Ole Petter Thangstad	Dr. scient Botany	Molecular studies of myrosinase in Brassicaceae
1993	Thrine L. M. Heggberget	Dr. scient Zoology	Reproductive strategy and feeding ecology of the Eurasian otter *Lutra lutra*.
1993	Kjetil Bevanger	Dr. scient Zoology	Avian interactions with utility structures, a biological approach.
1993	Kåre Haugan	Dr. scient Botany	Mutations in the replication control gene trfA of the broad host-range plasmid RK2
1994	Peder Fiske	Dr. scient Zoology	Sexual selection in the lekking great snipe (*Gallinago media*): Male mating success and female behaviour at the lek
1994	Kjell Inge Reitan	Dr. scient Botany	Nutritional effects of algae in first-feeding of marine fish larvae
1994	Nils Røv	Dr. scient Zoology	Breeding distribution, population status and regulation of breeding numbers in the northeast-Atlantic Great Cormorant *Phalacrocorax carbo carbo*
1994	Annette-Susanne Hoepfner	Dr. scient Botany	Tissue culture techniques in propagation and breeding of Red Raspberry (*Rubus idaeus* L.)
1994	Inga Elise Bruteig	Dr. scient Botany	Distribution, ecology and biomonitoring studies of epiphytic lichens on conifers
1994	Geir Johnsen	Dr. scient Botany	Light harvesting and utilization in marine phytoplankton: Species-specific and photoadaptive responses

1994	Morten Bakken	Dr. scient Zoology	Infanticidal behaviour and reproductive performance in relation to competition capacity among farmed silver fox vixens, *Vulpes vulpes*
1994	Arne Moksnes	Dr. philos Zoology	Host adaptations towards brood parasitism by the Cockoo
1994	Solveig Bakken	Dr. scient Botany	Growth and nitrogen status in the moss *Dicranum majus* Sm. as influenced by nitrogen supply
1994	Torbjørn Forseth	Dr. scient Zoology	Bioenergetics in ecological and life history studies of fishes.
1995	Olav Vadstein	Dr. philos Botany	The role of heterotrophic planktonic bacteria in the cycling of phosphorus in lakes: Phosphorus requirement, competitive ability and food web interactions
1995	Hanne Christensen	Dr. scient Zoology	Determinants of Otter *Lutra lutra* distribution in Norway: Effects of harvest, polychlorinated biphenyls (PCBs), human population density and competition with mink *Mustela vision*
1995	Svein Håkon Lorentsen	Dr. scient Zoology	Reproductive effort in the Antarctic Petrel *Thalassoica antarctica*; the effect of parental body size and condition
1995	Chris Jørgen Jensen	Dr. scient Zoology	The surface electromyographic (EMG) amplitude as an estimate of upper trapezius muscle activity
1995	Martha Kold Bakkevig	Dr. scient Zoology	The impact of clothing textiles and construction in a clothing system on thermoregulatory responses, sweat accumulation and heat transport
1995	Vidar Moen	Dr. scient Zoology	Distribution patterns and adaptations to light in newly introduced populations of *Mysis relicta* and constraints on Cladoceran and Char populations
1995	Hans Haavardsholm Blom	Dr. philos Botany	A revision of the *Schistidium apocarpum* complex in Norway and Sweden
1996	Jorun Skjærmo	Dr. scient Botany	Microbial ecology of early stages of cultivated marine fish; inpact fish-bacterial interactions on growth and survival of larvae
1996	Ola Ugedal	Dr. scient Zoology	Radiocesium turnover in freshwater fishes
1996	Ingibjørg Einarsdottir	Dr. scient Zoology	Production of Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus*): A study of some physiological and immunological responses to rearing routines
1996	Christina M. S. Pereira	Dr. scient Zoology	Glucose metabolism in salmonids: Dietary effects and hormonal regulation
1996	Jan Fredrik Børseth	Dr. scient Zoology	The sodium energy gradients in muscle cells of *Mytilus edulis* and the effects of organic xenobiotics
1996	Gunnar Henriksen	Dr. scient Zoology	Status of Grey seal *Halichoerus grypus* and Harbour seal *Phoca vitulina* in the Barents sea region
1997	Gunvor Øie	Dr. scient Botany	Eevalution of rotifer *Brachionus plicatilis* quality in early first feeding of turbot *Scophtalmus maximus* L. larvae
1997	Håkon Holien	Dr. scient Botany	Studies of lichens in spruce forest of Central Norway. Diversity, old growth species and the relationship to site and stand parameters
1997	Ole Reitan	Dr. scient Zoology	Responses of birds to habitat disturbance due to damming
1997	Jon Arne Grøttum	Dr. scient Zoology	Physiological effects of reduced water quality on fish in aquaculture

1997	Per Gustav Thingstad	Dr. scient Zoology	Birds as indicators for studying natural and human-induced variations in the environment, with special emphasis on the suitability of the Pied Flycatcher
1997	Torgeir Nygård	Dr. scient Zoology	Temporal and spatial trends of pollutants in birds in Norway: Birds of prey and Willow Grouse used as
1997	Signe Nybø	Dr. scient Zoology	Impacts of long-range transported air pollution on birds with particular reference to the dipper *Cinclus cinclus* in southern Norway
1997	Atle Wibe	Dr. scient Zoology	Identification of conifer volatiles detected by receptor neurons in the pine weevil (*Hylobius abietis*), analysed by gas chromatography linked to electrophysiology and to mass spectrometry
1997	Rolv Lundheim	Dr. scient Zoology	Adaptive and incidental biological ice nucleators
1997	Arild Magne Landa	Dr. scient Zoology	Wolverines in Scandinavia: ecology, sheep depredation and conservation
1997	Kåre Magne Nielsen	Dr. scient Botany	An evolution of possible horizontal gene transfer from plants to sail bacteria by studies of natural transformation in *Acinetobacter calcoacetius*
1997	Jarle Tufto	Dr. scient Zoology	Gene flow and genetic drift in geographically structured populations: Ecological, population genetic, and statistical models
1997	Trygve Hesthagen	Dr. philos Zoology	Population responses of Arctic charr (*Salvelinus alpinus* (L.)) and brown trout (*Salmo trutta* L.) to acidification in Norwegian inland waters
1997	Trygve Sigholt	Dr. philos Zoology	Control of Parr-smolt transformation and seawater tolerance in farmed Atlantic Salmon (*Salmo salar*) Effects of photoperiod, temperature, gradual seawater acclimation, NaCl and betaine in the diet
1997	Jan Østnes	Dr. scient Zoology	Cold sensation in adult and neonate birds
1998	Seethaledsumy Visvalingam	Dr. scient Botany	Influence of environmental factors on myrosinases and myrosinase-binding proteins
1998	Thor Harald Ringsby	Dr. scient Zoology	Variation in space and time: The biology of a House sparrow metapopulation
1998	Erling Johan Solberg	Dr. scient Zoology	Variation in population dynamics and life history in a Norwegian moose (*Alces alces*) population: consequences of harvesting in a variable environment
1998	Sigurd Mjøen Saastad	Dr. scient Botany	Species delimitation and phylogenetic relationships between the Sphagnum recurvum complex (Bryophyta): genetic variation and phenotypic plasticity
1998	Bjarte Mortensen	Dr. scient Botany	Metabolism of volatile organic chemicals (VOCs) in a head liver S9 vial equilibration system in vitro
1998	Gunnar Austrheim	Dr. scient Botany	Plant biodiversity and land use in subalpine grasslands. – A conservation biological approach
1998	Bente Gunnveig Berg	Dr. scient Zoology	Encoding of pheromone information in two related moth species
1999	Kristian Overskaug	Dr. scient Zoology	Behavioural and morphological characteristics in Northern Tawny Owls *Strix aluco*: An intra- and interspecific comparative approach
1999	Hans Kristen Stenøien	Dr. scient Botany	Genetic studies of evolutionary processes in various populations of nonvascular plants (mosses, liverworts and hornworts)
1999	Trond Arnesen	Dr. scient Botany	Vegetation dynamics following trampling and burning in the outlying haylands at Sølendet, Central Norway

1999	Ingvar Stenberg	Dr. scient Zoology	Habitat selection, reproduction and survival in the White-backed Woodpecker *Dendrocopos leucotos*
1999	Stein Olle Johansen	Dr. scient Botany	A study of driftwood dispersal to the Nordic Seas by dendrochronology and wood anatomical analysis
1999	Trina Falck Galloway	Dr. scient Zoology	Muscle development and growth in early life stages of the Atlantic cod (*Gadus morhua* L.) and Halibut (*Hippoglossus hippoglossus* L.)
1999	Marianne Giæver	Dr. scient Zoology	Population genetic studies in three gadoid species: blue whiting (*Micromisistius poutassou*), haddock (*Melanogrammus aeglefinus*) and cod (*Gadus morhua*) in the North-East Atlantic
1999	Hans Martin Hanslin	Dr. scient Botany	The impact of environmental conditions of density dependent performance in the boreal forest bryophytes *Dicranum majus*, *Hylocomium splendens*, *Plagiochila asplenigides*, *Ptilium crista-castrensis* and *Rhytidiadelphus lokeus*
1999	Ingrid Bysveen Mjølnerød	Dr. scient Zoology	Aspects of population genetics, behaviour and performance of wild and farmed Atlantic salmon (*Salmo salar*) revealed by molecular genetic techniques
1999	Else Berit Skagen	Dr. scient Botany	The early regeneration process in protoplasts from *Brassica napus* hypocotyls cultivated under various g-forces
1999	Stein-Are Sæther	Dr. philos Zoology	Mate choice, competition for mates, and conflicts of interest in the Lekking Great Snipe
1999	Katrine Wangen Rustad	Dr. scient Zoology	Modulation of glutamatergic neurotransmission related to cognitive dysfunctions and Alzheimer's disease
1999	Per Terje Smiseth	Dr. scient Zoology	Social evolution in monogamous families:
1999	Gunnbjørn Bremset	Dr. scient Zoology	Young Atlantic salmon (*Salmo salar* L.) and Brown trout (*Salmo trutta* L.) inhabiting the deep pool habitat, with special reference to their habitat use, habitat preferences and competitive interactions
1999	Frode Ødegaard	Dr. scient Zoology	Host specificity as a parameter in estimates of arthropod species richness
1999	Sonja Andersen	Dr. scient Zoology	Expressional and functional analyses of human, secretory phospholipase A2
2000	Ingrid Salvesen	Dr. scient Botany	Microbial ecology in early stages of marine fish: Development and evaluation of methods for microbial management in intensive larviculture
2000	Ingar Jostein Øien	Dr. scient Zoology	The Cuckoo (*Cuculus canorus*) and its host: adaptions and counteradaptions in a coevolutionary arms race
2000	Pavlos Makridis	Dr. scient Botany	Methods for the microbial control of live food used for the rearing of marine fish larvae
2000	Sigbjørn Stokke	Dr. scient Zoology	Sexual segregation in the African elephant (*Loxodonta africana*)
2000	Odd A. Gulseth	Dr. philos Zoology	Seawater tolerance, migratory behaviour and growth of Charr, (*Salvelinus alpinus*), with emphasis on the high Arctic Dieset charr on Spitsbergen, Svalbard
2000	Pål A. Olsvik	Dr. scient Zoology	Biochemical impacts of Cd, Cu and Zn on brown trout (*Salmo trutta*) in two mining-contaminated rivers in Central Norway
2000	Sigurd Einum	Dr. scient Zoology	Maternal effects in fish: Implications for the evolution of breeding time and egg size
2001	Jan Ove Evjemo	Dr. scient Zoology	Production and nutritional adaptation of the brine shrimp *Artemia* sp. as live food organism for larvae of marine cold water fish species

2001	Olga Hilmo	Dr. scient Botany	Lichen response to environmental changes in the managed boreal forest systems
2001	Ingebrigt Uglem	Dr. scient Zoology	Male dimorphism and reproductive biology in corkwing wrasse (*Symphodus melops* L.)
2001	Bård Gunnar Stokke	Dr. scient Zoology	Coevolutionary adaptations in avian brood parasites and their hosts
2002	Ronny Aanes	Dr. scient Zoology	Spatio-temporal dynamics in Svalbard reindeer (*Rangifer tarandus platyrhynchus*)
2002	Mariann Sandsund	Dr. scient Zoology	Exercise- and cold-induced asthma. Respiratory and thermoregulatory responses
2002	Dag-Inge Øien	Dr. scient Botany	Dynamics of plant communities and populations in boreal vegetation influenced by scything at Sølendet, Central Norway
2002	Frank Rosell	Dr. scient Zoology	The function of scent marking in beaver (*Castor fiber*)
2002	Janne Østvang	Dr. scient Botany	The Role and Regulation of Phospholipase A_2 in Monocytes During Atherosclerosis Development
2002	Terje Thun	Dr. philos Biology	Dendrochronological constructions of Norwegian conifer chronologies providing dating of historical material
2002	Birgit Hafjeld Borgen	Dr. scient Biology	Functional analysis of plant idioblasts (Myrosin cells) and their role in defense, development and growth
2002	Bård Øyvind Solberg	Dr. scient Biology	Effects of climatic change on the growth of dominating tree species along major environmental gradients
2002	Per Winge	Dr. scient Biology	The evolution of small GTP binding proteins in cellular organisms. Studies of RAC GTPases in *Arabidopsis thaliana* and the Ral GTPase from *Drosophila melanogaster*
2002	Henrik Jensen	Dr. scient Biology	Causes and consequences of individual variation in fitness-related traits in house sparrows
2003	Jens Rohloff	Dr. philos Biology	Cultivation of herbs and medicinal plants in Norway – Essential oil production and quality control
2003	Åsa Maria O. Espmark Wibe	Dr. scient Biology	Behavioural effects of environmental pollution in threespine stickleback *Gasterosteus aculeatur* L.
2003	Dagmar Hagen	Dr. scient Biology	Assisted recovery of disturbed arctic and alpine vegetation – an integrated approach
2003	Bjørn Dahle	Dr. scient Biology	Reproductive strategies in Scandinavian brown bears
2003	Cyril Lebogang Taolo	Dr. scient Biology	Population ecology, seasonal movement and habitat use of the African buffalo (*Syncerus caffer*) in Chobe National Park, Botswana
2003	Marit Stranden	Dr. scient Biology	Olfactory receptor neurones specified for the same odorants in three related Heliothine species (*Helicoverpa armigera, Helicoverpa assulta* and *Heliothis virescens*)
2003	Kristian Hassel	Dr. scient Biology	Life history characteristics and genetic variation in an expanding species, *Pogonatum dentatum*
2003	David Alexander Rae	Dr. scient Biology	Plant- and invertebrate-community responses to species interaction and microclimatic gradients in alpine and Artic environments
2003	Åsa A Borg	Dr. scient Biology	Sex roles and reproductive behaviour in gobies and guppies: a female perspective
2003	Eldar Åsgard Bendiksen	Dr. scient Biology	Environmental effects on lipid nutrition of farmed Atlantic salmon (*Salmo salar* L.) parr and smolt
2004	Torkild Bakken	Dr. scient Biology	A revision of Nereidinae (Polychaeta, Nereididae)

2004	Ingar Pareliussen	Dr. scient Biology	Natural and Experimental Tree Establishment in a Fragmented Forest, Ambohitantely Forest Reserve, Madagascar
2004	Tore Brembu	Dr. scient Biology	Genetic, molecular and functional studies of RAC GTPases and the WAVE-like regulatory protein complex in *Arabidopsis thaliana*
2004	Liv S. Nilsen	Dr. scient Biology	Coastal heath vegetation on central Norway; recent past, present state and future possibilities
2004	Hanne T. Skiri	Dr. scient Biology	Olfactory coding and olfactory learning of plant odours in heliothine moths. An anatomical, physiological and behavioural study of three related species (*Heliothis virescens, Helicoverpa armigera* and *Helicoverpa assulta*)
2004	Lene Østby	Dr. scient Biology	Cytochrome P4501A (CYP1A) induction and DNA adducts as biomarkers for organic pollution in the natural environment
2004	Emmanuel J. Gerreta	Dr. philos Biology	The Importance of Water Quality and Quantity in the Tropical Ecosystems, Tanzania
2004	Linda Dalen	Dr. scient Biology	Dynamics of Mountain Birch Treelines in the Scandes Mountain Chain, and Effects of Climate Warming
2004	Lisbeth Mehli	Dr. scient Biology	Polygalacturonase-inhibiting protein (PGIP) in cultivated strawberry (*Fragaria* x *ananassa*): characterisation and induction of the gene following fruit infection by *Botrytis cinerea*
2004	Børge Moe	Dr. scient Biology	Energy-Allocation in Avian Nestlings Facing Short-Term Food Shortage
2005	Matilde Skogen Chauton	Dr. scient Biology	Metabolic profiling and species discrimination from High-Resolution Magic Angle Spinning NMR analysis of whole-cell samples
2005	Sten Karlsson	Dr. scient Biology	Dynamics of Genetic Polymorphisms
2005	Terje Bongard	Dr. scient Biology	Life History strategies, mate choice, and parental investment among Norwegians over a 300-year period
2005	Tonette Røstelien	PhD Biology	Functional characterisation of olfactory receptor neurone types in heliothine moths
2005	Erlend Kristiansen	Dr. scient Biology	Studies on antifreeze proteins
2005	Eugen G. Sørmo	Dr. scient Biology	Organochlorine pollutants in grey seal (*Halichoerus grypus*) pups and their impact on plasma thyroid hormone and vitamin A concentrations
2005	Christian Westad	Dr. scient Biology	Motor control of the upper trapezius
2005	Lasse Mork Olsen	PhD Biology	Interactions between marine osmo- and phagotrophs in different physicochemical environments
2005	Åslaug Viken	PhD Biology	Implications of mate choice for the management of small populations
2005	Ariaya Hymete Sahle Dingle	PhD Biology	Investigation of the biological activities and chemical constituents of selected *Echinops* spp. growing in Ethiopia
2005	Anders Gravbrøt Finstad	PhD Biology	Salmonid fishes in a changing climate: The winter challenge
2005	Shimane Washington Makabu	PhD Biology	Interactions between woody plants, elephants and other browsers in the Chobe Riverfront, Botswana
2005	Kjartan Østbye	Dr. scient Biology	The European whitefish *Coregonus lavaretus* (L.) species complex: historical contingency and adaptive radiation

2006	Kari Mette Murvoll	PhD Biology	Levels and effects of persistent organic pollutans (POPs) in seabirds, Retinoids and α-tocopherol – potential biomakers of POPs in birds?
2006	Ivar Herfindal	Dr. scient Biology	Life history consequences of environmental variation along ecological gradients in northern ungulates
2006	Nils Egil Tokle	PhD Biology	Are the ubiquitous marine copepods limited by food or predation? Experimental and field-based studies with main focus on *Calanus finmarchicus*
2006	Jan Ove Gjershaug	Dr. philos Biology	Taxonomy and conservation status of some booted eagles in south-east Asia
2006	Jon Kristian Skei	Dr. scient Biology	Conservation biology and acidification problems in the breeding habitat of amphibians in Norway
2006	Johanna Järnegren	PhD Biology	*Acesta oophaga* and *Acesta excavata* – a study of hidden biodiversity
2006	Bjørn Henrik Hansen	PhD Biology	Metal-mediated oxidative stress responses in brown trout (*Salmo trutta*) from mining contaminated rivers in Central Norway
2006	Vidar Grøtan	PhD Biology	Temporal and spatial effects of climate fluctuations on population dynamics of vertebrates
2006	Jafari R Kideghesho	PhD Biology	Wildlife conservation and local land use conflicts in Western Serengeti Corridor, Tanzania
2006	Anna Maria Billing	PhD Biology	Reproductive decisions in the sex role reversed pipefish *Syngnathus typhle*: when and how to invest in reproduction
2006	Henrik Pärn	PhD Biology	Female ornaments and reproductive biology in the bluethroat
2006	Anders J. Fjellheim	PhD Biology	Selection and administration of probiotic bacteria to marine fish larvae
2006	P. Andreas Svensson	PhD Biology	Female coloration, egg carotenoids and reproductive success: gobies as a model system
2007	Sindre A. Pedersen	PhD Biology	Metal binding proteins and antifreeze proteins in the beetle *Tenebrio molitor* - a study on possible competition for the semi-essential amino acid cysteine
2007	Kasper Hancke	PhD Biology	Photosynthetic responses as a function of light and temperature: Field and laboratory studies on marine microalgae
2007	Tomas Holmern	PhD Biology	Bushmeat hunting in the western Serengeti: Implications for community-based conservation
2007	Kari Jørgensen	PhD Biology	Functional tracing of gustatory receptor neurons in the CNS and chemosensory learning in the moth *Heliothis virescens*
2007	Stig Ulland	PhD Biology	Functional Characterisation of Olfactory Receptor Neurons in the Cabbage Moth, (*Mamestra brassicae* L.) (Lepidoptera, Noctuidae). Gas Chromatography Linked to Single Cell Recordings and Mass Spectrometry
2007	Snorre Henriksen	PhD Biology	Spatial and temporal variation in herbivore resources at northern latitudes
2007	Roelof Frans May	PhD Biology	Spatial Ecology of Wolverines in Scandinavia
2007	Vedasto Gabriel Ndibalema	PhD Biology	Demographic variation, distribution and habitat use between wildebeest sub-populations in the Serengeti National Park, Tanzania
2007	Julius William Nyahongo	PhD Biology	Depredation of Livestock by wild Carnivores and Illegal Utilization of Natural Resources by Humans in the Western Serengeti, Tanzania

2007	Shombe Ntaraluka Hassan	PhD Biology	Effects of fire on large herbivores and their forage resources in Serengeti, Tanzania
2007	Per-Arvid Wold	PhD Biology	Functional development and response to dietary treatment in larval Atlantic cod *(Gadus morhua* L.) Focus on formulated diets and early weaning
2007	Anne Skjetne Mortensen	PhD Biology	Toxicogenomics of Aryl Hydrocarbon- and Estrogen Receptor Interactions in Fish: Mechanisms and Profiling of Gene Expression Patterns in Chemical Mixture Exposure Scenarios
2008	Brage Bremset Hansen	PhD Biology	The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and its food base: plant-herbivore interactions in a high-arctic ecosystem
2008	Jiska van Dijk	PhD Biology	Wolverine foraging strategies in a multiple-use landscape
2008	Flora John Magige	PhD Biology	The ecology and behaviour of the Masai Ostrich (*Struthio camelus massaicus*) in the Serengeti Ecosystem, Tanzania
2008	Bernt Rønning	PhD Biology	Sources of inter- and intra-individual variation in basal metabolic rate in the zebra finch, *Taeniopygia guttata*
2008	Sølvi Wehn	PhD Biology	Biodiversity dynamics in semi-natural mountain landscapes - A study of consequences of changed agricultural practices in Eastern Jotunheimen
2008	Trond Moxness Kortner	PhD Biology	The Role of Androgens on previtellogenic oocyte growth in Atlantic cod (*Gadus morhua*): Identification and patterns of differentially expressed genes in relation to Stereological Evaluations
2008	Katarina Mariann Jørgensen	Dr. scient Biology	The role of platelet activating factor in activation of growth arrested keratinocytes and re-epithelialisation
2008	Tommy Jørstad	PhD Biology	Statistical Modelling of Gene Expression Data
2008	Anna Kusnierczyk	PhD Biology	*Arabidopsis thaliana* Responses to Aphid Infestation
2008	Jussi Evertsen	PhD Biology	Herbivore sacoglossans with photosynthetic chloroplasts
2008	John Eilif Hermansen	PhD Biology	Mediating ecological interests between locals and globals by means of indicators. A study attributed to the asymmetry between stakeholders of tropical forest at Mt. Kilimanjaro, Tanzania
2008	Ragnhild Lyngved	PhD Biology	Somatic embryogenesis in *Cyclamen persicum.* Biological investigations and educational aspects of cloning
2008	Line Elisabeth Sundt-Hansen	PhD Biology	Cost of rapid growth in salmonid fishes
2008	Line Johansen	PhD Biology	Exploring factors underlying fluctuations in white clover populations – clonal growth, population structure and spatial distribution
2009	Astrid Jullumstrø Feuerherm	PhD Biology	Elucidation of molecular mechanisms for pro-inflammatory phospholipase A2 in chronic disease
2009	Pål Kvello	PhD Biology	Neurons forming the network involved in gustatory coding and learning in the moth *Heliothis virescens:* Physiological and morphological characterisation, and integration into a standard brain atlas
2009	Trygve Devold Kjellsen	PhD Biology	Extreme Frost Tolerance in Boreal Conifers
2009	Johan Reinert Vikan	PhD Biology	Coevolutionary interactions between common cuckoos *Cuculus canorus* and *Fringilla* finches

2009	Zsolt Volent	PhD Biology	Remote sensing of marine environment: Applied surveillance with focus on optical properties of phytoplankton, coloured organic matter and suspended matter
2009	Lester Rocha	PhD Biology	Functional responses of perennial grasses to simulated grazing and resource availability
2009	Dennis Ikanda	PhD Biology	Dimensions of a Human-lion conflict: Ecology of human predation and persecution of African lions (*Panthera leo*) in Tanzania
2010	Huy Quang Nguyen	PhD Biology	Egg characteristics and development of larval digestive function of cobia (*Rachycentron canadum*) in response to dietary treatments - Focus on formulated diets
2010	Eli Kvingedal	PhD Biology	Intraspecific competition in stream salmonids: the impact of environment and phenotype
2010	Sverre Lundemo	PhD Biology	Molecular studies of genetic structuring and demography in *Arabidopsis* from Northern Europe
2010	Iddi Mihijai Mfunda	PhD Biology	Wildlife Conservation and People's livelihoods: Lessons Learnt and Considerations for Improvements. The Case of Serengeti Ecosystem, Tanzania
2010	Anton Tinchov Antonov	PhD Biology	Why do cuckoos lay strong-shelled eggs? Tests of the puncture resistance hypothesis
2010	Anders Lyngstad	PhD Biology	Population Ecology of *Eriophorum latifolium*, a Clonal Species in Rich Fen Vegetation
2010	Hilde Færevik	PhD Biology	Impact of protective clothing on thermal and cognitive responses
2010	Ingerid Brænne Arbo	PhD Medical technology	Nutritional lifestyle changes – effects of dietary carbohydrate restriction in healthy obese and overweight humans
2010	Yngvild Vindenes	PhD Biology	Stochastic modeling of finite populations with individual heterogeneity in vital parameters
2010	Hans-Richard Brattbakk	PhD Medical technology	The effect of macronutrient composition, insulin stimulation, and genetic variation on leukocyte gene expression and possible health benefits
2011	Geir Hysing Bolstad	PhD Biology	Evolution of Signals: Genetic Architecture, Natural Selection and Adaptive Accuracy
2011	Karen de Jong	PhD Biology	Operational sex ratio and reproductive behaviour in the two-spotted goby (*Gobiusculus flavescens*)
2011	Ann-Iren Kittang	PhD Biology	*Arabidopsis thaliana* L. adaptation mechanisms to microgravity through the EMCS MULTIGEN-2 experiment on the ISS: The science of space experiment integration and adaptation to simulated microgravity
2011	Aline Magdalena Lee	PhD Biology	Stochastic modeling of mating systems and their effect on population dynamics and genetics
2011	Christopher Gravningen Sørmo	PhD Biology	Rho GTPases in Plants: Structural analysis of ROP GTPases; genetic and functional studies of MIRO GTPases in Arabidopsis thaliana
2011	Grethe Robertsen	PhD Biology	Relative performance of salmonid phenotypes across environments and competitive intensities
2011	Line-Kristin Larsen	PhD Biology	Life-history trait dynamics in experimental populations of guppy (*Poecilia reticulata*): the role of breeding regime and captive environment
2011	Maxim A. K. Teichert	PhD Biology	Regulation in Atlantic salmon (*Salmo salar*): The interaction between habitat and density
2011	Torunn Beate Hancke	PhD Biology	Use of Pulse Amplitude Modulated (PAM) Fluorescence and Bio-optics for Assessing Microalgal Photosynthesis and Physiology

2011	Sajeda Begum	PhD Biology	Brood Parasitism in Asian Cuckoos: Different Aspects of Interactions between Cuckoos and their Hosts in Bangladesh
2011	Kari J. K. Attramadal	PhD Biology	Water treatment as an approach to increase microbial control in the culture of cold water marine larvae
2011	Camilla Kalvatn Egset	PhD Biology	The Evolvability of Static Allometry: A Case Study
2011	AHM Raihan Sarker	PhD Biology	Conflict over the conservation of the Asian elephant (*Elephas maximus*) in Bangladesh
2011	Gro Dehli Villanger	PhD Biology	Effects of complex organohalogen contaminant mixtures on thyroid hormone homeostasis in selected arctic marine mammals
2011	Kari Bjørneraas	PhD Biology	Spatiotemporal variation in resource utilisation by a large herbivore, the moose
2011	John Odden	PhD Biology	The ecology of a conflict: Eurasian lynx depredation on domestic sheep
2011	Simen Pedersen	PhD Biology	Effects of native and introduced cervids on small mammals and birds
2011	Mohsen Falahati-Anbaran	PhD Biology	Evolutionary consequences of seed banks and seed dispersal in *Arabidopsis*
2012	Jakob Hønborg Hansen	PhD Biology	Shift work in the offshore vessel fleet: circadian rhythms and cognitive performance
2012	Elin Noreen	PhD Biology	Consequences of diet quality and age on life-history traits in a small passerine bird
2012	Irja Ida Ratikainen	PhD Biology	Foraging in a variable world: adaptations to stochasticity
2012	Aleksander Handå	PhD Biology	Cultivation of mussels (*Mytilus edulis):* Feed requirements, storage and integration with salmon (*Salmo salar*) farming
2012	Morten Kraabøl	PhD Biology	Reproductive and migratory challenges inflicted on migrant brown trout (*Salmo trutta* L.) in a heavily modified river
2012	Jisca Huisman	PhD Biology	Gene flow and natural selection in Atlantic salmon
2012	Maria Bergvik	PhD Biology	Lipid and astaxanthin contents and biochemical post-harvest stability in *Calanus finmarchicus*
2012	Bjarte Bye Løfaldli	PhD Biology	Functional and morphological characterization of central olfactory neurons in the model insect *Heliothis virescens*.
2012	Karen Marie Hammer	PhD Biology	Acid-base regulation and metabolite responses in shallow- and deep-living marine invertebrates during environmental hypercapnia
2012	Øystein Nordrum Wiggen	PhD Biology	Optimal performance in the cold
2012	Robert Dominikus Fyumagwa	Dr. Philos Biology	Anthropogenic and natural influence on disease prevalence at the human –livestock-wildlife interface in the Serengeti ecosystem, Tanzania
2012	Jenny Bytingsvik	PhD Biology	Organohalogenated contaminants (OHCs) in polar bear mother-cub pairs from Svalbard, Norway. Maternal transfer, exposure assessment and thyroid hormone disruptive effects in polar bear cubs
2012	Christer Moe Rolandsen	PhD Biology	The ecological significance of space use and movement patterns of moose in a variable environment
2012	Erlend Kjeldsberg Hovland	PhD Biology	Bio-optics and Ecology in *Emiliania huxleyi* Blooms: Field and Remote Sensing Studies in Norwegian Waters

2012	Lise Cats Myhre	PhD Biology	Effects of the social and physical environment on mating behaviour in a marine fish
2012	Tonje Aronsen	PhD Biology	Demographic, environmental and evolutionary aspects of sexual selection
2012	Bin Liu	PhD Biology	Molecular genetic investigation of cell separation and cell death regulation in *Arabidopsis thaliana*
2013	Jørgen Rosvold	PhD Biology	Ungulates in a dynamic and increasingly human dominated landscape – A millennia-scale perspective
2013	Pankaj Barah	PhD Biology	Integrated Systems Approaches to Study Plant Stress Responses
2013	Marit Linnerud	PhD Biology	Patterns in spatial and temporal variation in population abundances of vertebrates
2013	Xinxin Wang	PhD Biology	Integrated multi-trophic aquaculture driven by nutrient wastes released from Atlantic salmon (*Salmo salar*) farming
2013	Ingrid Ertshus Mathisen	PhD Biology	Structure, dynamics, and regeneration capacity at the sub-arctic forest-tundra ecotone of northern Norway and Kola Peninsula, NW Russia
2013	Anders Foldvik	PhD Biology	Spatial distributions and productivity in salmonid populations
2013	Anna Marie Holand	PhD Biology	Statistical methods for estimating intra- and inter-population variation in genetic diversity
2013	Anna Solvang Båtnes	PhD Biology	Light in the dark – the role of irradiance in the high Arctic marine ecosystem during polar night
2013	Sebastian Wacker	PhD Biology	The dynamics of sexual selection: effects of OSR, density and resource competition in a fish
2013	Cecilie Miljeteig	PhD Biology	Phototaxis in *Calanus finmarchicus* – light sensitivity and the influence of energy reserves and oil exposure
2013	Ane Kjersti Vie	PhD Biology	Molecular and functional characterisation of the IDA family of signalling peptides in *Arabidopsis thaliana*
2013	Marianne Nymark	PhD Biology	Light responses in the marine diatom *Phaeodactylum tricornutum*
2014	Jannik Schultner	PhD Biology	Resource Allocation under Stress - Mechanisms and Strategies in a Long-Lived Bird
2014	Craig Ryan Jackson	PhD Biology	Factors influencing African wild dog *(Lycaon pictus*) habitat selection and ranging behaviour: conservation and management implications
2014	Aravind Venkatesan	PhD Biology	Application of Semantic Web Technology to establish knowledge management and discovery in the Life Sciences
2014	Kristin Collier Valle	PhD Biology	Photoacclimation mechanisms and light responses in marine micro- and macroalgae
2014	Michael Puffer	PhD Biology	Effects of rapidly fluctuating water levels on juvenile Atlantic salmon (*Salmo salar* L.)
2014	Gundula S. Bartzke	PhD Biology	Effects of power lines on moose *(Alces alces)* habitat selection, movements and feeding activity
2014	Eirin Marie Bjørkvoll	PhD Biology	Life-history variation and stochastic population dynamics in vertebrates
2014	Håkon Holand	PhD Biology	The parasite *Syngamus trachea* in a metapopulation of house sparrows
2014	Randi Magnus Sommerfelt	PhD Biology	Molecular mechanisms of inflammation – a central role for cytosolic phospholiphase A2
2014	Espen Lie Dahl	PhD Biology	Population demographics in white-tailed eagle at an on-shore wind farm area in coastal Norway

2014	Anders Øverby	PhD Biology	Functional analysis of the action of plant isothiocyanates: cellular mechanisms and in vivo role in plants, and anticancer activity
2014	Kamal Prasad Acharya	PhD Biology	Invasive species: Genetics, characteristics and trait variation along a latitudinal gradient.
2014	Ida Beathe Øverjordet	PhD Biology	Element accumulation and oxidative stress variables in Arctic pelagic food chains: *Calanus*, little auks (*Alle alle*) and black-legged kittiwakes (*Rissa tridactyla*)
2014	Kristin Møller Gabrielsen	PhD Biology	Target tissue toxicity of the thyroid hormone system in two species of arctic mammals carrying high loads of organohalogen contaminants
2015	Gine Roll Skjervø	Dr. philos Biology	Testing behavioral ecology models with historical individual-based human demographic data from Norway
2015	Nils Erik Gustaf Forsberg	PhD Biology	Spatial and Temporal Genetic Structure in Landrace Cereals
2015	Leila Alipanah	PhD Biology	Integrated analyses of nitrogen and phosphorus deprivation in the diatoms *Phaeodactylum tricornutum* and *Seminavis robusta*
2015	Javad Najafi	PhD Biology	Molecular investigation of signaling components in sugar sensing and defense in *Arabidopsis thaliana*
2015	Bjørnar Sporsheim	PhD Biology	Quantitative confocal laser scanning microscopy: optimization of in vivo and in vitro analysis of intracellular transport
2015	Magni Olsen Kyrkjeeide	PhD Biology	Genetic variation and structure in peatmosses (*Sphagnum*)
2015	Keshuai Li	PhD Biology	Phospholipids in Atlantic cod (*Gadus morhua* L.) larvae rearing: Incorporation of DHA in live feed and larval phospholipids and the metabolic capabilities of larvae for the de novo synthesis
2015	Ingvild Fladvad Størdal	PhD Biology	The role of the copepod *Calanus finmarchicus* in affecting the fate of marine oil spills
2016	Thomas Kvalnes	PhD Biology	Evolution by natural selection in age-structured populations in fluctuating environments
2016	Øystein Leiknes	PhD Biology	The effect of nutrition on important life-history traits in the marine copepod *Calanus finmarchicus*
2016	Johan Henrik Hårdensson Berntsen	PhD Biology	Individual variation in survival: The effect of incubation temperature on the rate of physiological ageing in a small passerine bird
2016	Marianne Opsahl Olufsen	PhD Biology	Multiple environmental stressors: Biological interactions between parameters of climate change and perfluorinated alkyl substances in fish
2016	Rebekka Varne	PhD Biology	Tracing the fate of escaped cod (*Gadus morhua* L.) in a Norwegian fjord system
2016	Anette Antonsen Fenstad	PhD Biology	Pollutant Levels, Antioxidants and Potential Genotoxic Effects in Incubating Female Common Eiders (*Somateria mollissima*)
2016	Wilfred Njama Marealle	PhD Biology	Ecology, Behaviour and Conservation Status of Masai Giraffe (*Giraffa camelopardalis tippelskirchi*) in Tanzania
2016	Ingunn Nilssen	PhD Biology	Integrated Enviromental Mapping and Monitoring: A Methodological approach for end users.
2017	Konika Chawla	PhD Biology	Discovering, analysing and taking care of knowledge.
2017	Øystein Hjorthol Opedal	PhD Biology	The Evolution of Herkogamy: Pollinator Reliability, Natural Selection, and Trait Evolvability.

2017	Ane Marlene Myhre	PhD Biology	Effective size of density dependent populations in fluctuating environments
2017	Emmanuel Hosiana Masenga	PhD Biology	Behavioural Ecology of Free-ranging and Reintroduced African Wild Dog (*Lycaon pictus*) Packs in the Serengeti Ecosystem, Tanzania
2017	Xiaolong Lin	PhD Biology	Systematics and evolutionary history of *Tanytarsus* van der Wulp, 1874 (Diptera: Chironomidae)
2017	Emmanuel Clamsen Mmassy	PhD Biology	Ecology and Conservation Challenges of the Kori bustard in the Serengeti National Park
2017	Richard Daniel Lyamuya	PhD Biology	Depredation of Livestock by Wild Carnivores in the Eastern Serengeti Ecosystem, Tanzania
2017	Katrin Hoydal	PhD Biology	Levels and endocrine disruptive effects of legacy POPs and their metabolites in long-finned pilot whales of the Faroe Islands
2017	Berit Glomstad	PhD Biology	Adsorption of phenanthrene to carbon nanotubes and its influence on phenanthrene bioavailability/toxicity in aquatic organism
2017	Øystein Nordeide Kielland	PhD Biology	Sources of variation in metabolism of an aquatic ectotherm
2017	Narjes Yousefi	PhD Biology	Genetic divergence and speciation in northern peatmosses (*Sphagnum*)
2018	Signe Christensen-Dalgaard	PhD Biology	Drivers of seabird spatial ecology - implications for development of offshore wind-power in Norway
2018	Janos Urbancsok	PhD Biology	Endogenous biological effects induced by externally supplemented glucosinolate hydrolysis products (GHPs) on *Arabidopsis thaliana*
2018	Alice Mühlroth	PhD Biology	The influence of phosphate depletion on lipid metabolism of microalgae
2018	Franco Peniel Mbise	PhD Biology	Human-Carnivore Coexistence and Conflict in the Eastern Serengeti, Tanzania
2018	Stine Svalheim Markussen	PhD Biology	Causes and consequences of intersexual life history variation in a harvested herbivore population
2018	Mia Vedel Sørensen	PhD Biology	Carbon budget consequences of deciduous shrub expansion in alpine tundra ecosystems
2018	Hanna Maria Kauko	PhD Biology	Light response and acclimation of microalgae in a changing Arctic
2018	Erlend I. F. Fossen	PhD Biology	Trait evolvability: effects of thermal plasticity and genetic correlations among traits
2019	Peter Sjolte Ranke	PhD Biology	Demographic and genetic and consequences of dispersal in house sparrows
2019	Mathilde Le Moullec	PhD Biology	Spatiotemporal variation in abundance of key tundra species: from local heterogeneity to large-scale synchrony
2019	Endre Grüner Ofstad	PhD Biology	Causes and consequences of variation in resource use and social structure in ungulates
2019	Yang Jin	PhD Biology	Development of lipid metabolism in early life stage of Atlantic salmon (*Salmo salar*)
2019	Elena Albertsen	PhD Biology	Evolution of floral traits: from ecological contex to functional integration
2019	Mominul Islam Nahid	PhD Biology	Interaction between two Asian cuckoos and their hosts in Bangladesh
2019	Knut Jørgen Egelie	PhD Biology	Management of intellectual property in university-industry collaborations – public access to and control of knowledge
2019	Thomas Ray Haaland	PhD Biology	Adaptive responses to enviromental stochasticity on different evolutionary time-scales

2019	Kwaslema Malle Hariohay	PhD Biology	Human wildlife interactions in the Ruaha-Rungwa Ecosystem, Central Tanzania
2019	Mari Engvig Løseth	PhD Biology	Exposure and effects of emerging and legacy organic pollutants in white-tailed eagle (*Haliaeetis albicilla*) nestlings
2019	Joseph Mbyati Mukeka	PhD Biology	Human-Wildlife Conflicts and Compensation for Losses in Kenya: Dynamics, Characteristics and Correlates
2019	Helene Løvstrand Svarva	PhD Biology	Dendroclimatology in southern Norway: tree rings, demography and climate
2019	Nathalie Briels	PhD Biology	Exposure and effects of legacy and emerging organic pollutants in developing birds – Laboratory and field studies
2019	Anders L.Kolstad	PhD Biology	Moose browsing effects on boreal production forests – implications for ecosystems and human society
2019	Bart Peeters	PhD Biology	Population dynamics under climate change ad harvesting: Results from the high Arctic Svalbard reindeer
2019	Emma-Liina Marjakangas	PhD Biology	Understanding species interactions in the tropics: dynamics within and between trophic levels
2019	Alex Kojo Datsomor	PhD Biology	The molecular basis of long chain polyunsaturated fatty acid (LC-PUFA) biosynthesis in Atlantic salmon (*Salmo salar L*): In vivo functions, functional redundancy and transcriptional regulation of LC-PUFA biosynthetic enzymes
2020	Ingun Næve	PhD Biology	Development of non-invasive methods using ultrasound technology in monitoring of Atlantic salmon (*Salmo salar*) production and reproduction
2020	Rachael Morgan	PhD Biology	Physiological plasticity and evolution of thermal performance in zebrafish
2020	Mahsa Jalili	PhD Biology	Effects of different dietary ingredients on the immune responses and antioxidant status in Atlantic salmon (*Salmo salar L.):* possible nutriomics approaches
2020	Haiqing Wang	PhD Biology	Utilization of the polychaete *Hediste diversicolor* (O.F. Millier, 1776) in recycling waste nutrients from land-based fish farms for value adding applications'
2020	Louis Hunninck	PhD Biology	Physiological and behavioral adaptations of impala to anthropogenic disturbances in the Serengeti ecosystems
2020	Kate Layton-Matthews	PhD Biology	Demographic consequences of rapid climate change and density dependence in migratory Arctic geese
2020	Amit Kumar Sharma	PhD Biology	Genome editing of marine algae: Technology development and use of the CRISPR/Cas9 system for studies of light harvesting complexes and regulation of phosphate homeostasis
2020	Lars Rød-Eriksen	PhD Biology	Drivers of change in meso-carnivore distributions in a northern ecosystem
2020	Lone Sunniva Jevne	PhD Biology	Development and dispersal of salmon lice (*Lepeophtheirus salmonis Krøyer*, 1837) in commercial salmon farming localities
2020	Sindre Håvarstein Eldøy	PhD Biology	The influence of physiology, life history and environmental conditions on the marine migration patters of sea trout
2020	Vasundra Touré	PhD Biology	Improving the FAIRness of causal interactions in systems biology: data curation and standardisation to support systems modelling applications

2020	Silje Forbord	PhD Biology	Cultivation of *Saccharina latissima* (Phaeophyceae) in temperate marine waters; nitrogen uptake kinetics, growth characteristics and chemical composition
2020	Jørn Olav Løkken	PhD Biology	Change in vegetation composition and growth in the forest-tundra ecotone – effects of climate warming and herbivory
2020	Kristin Odden Nystuen	PhD Biology	Drivers of plant recruitment in alpine vegetation
2021	Sam Perrin	PhD Biology	Freshwater Fish Community Responses to Climate Change and Invasive Species
2021	Lara Veylit	PhD Biology	Causes and consequences of body growth variation in hunted wild boar populations
2021	Semona Issa	PhD Biology	Combined effects of environmental variation and pollution on zooplankton life history and population dynamics
2021	Monica Shilereyo	PhD Biology	Small Mammal Population Ecology and Ectoparasite Load: Assessing Impacts of Land Use and Rainfall Seasonality in the Serengeti Ecosystem, Tanzania
2021	Vanessa Bieker	PhD Biology	Using historical herbarium specimens to elucidate the evolutionary genomics of plant invasion
2021	Håkon Austad Langberg	PhD Biology	Fate and transport of forever chemicals in the aquatic environment: Partitioning and biotransformation of mixtures of Per- and Polyfluoroalkyl Substances (PFAS) from different point sources and resulting concentrations in biota
2021	Julie Renberg	PhD Biology	Muscular and metabolic load and manual function when working in the cold
2021	Olena Meleshko	PhD Biology	Gene flow and genome evolution on peatmosses (*Sphagnum*)
2021	Essa Ahsan Khan	PhD Biology	Systems toxicology approach for evaluating the effects of contaminants on fish ovarian development and reproductive endocrine physiology:A combination of field-, in vivo and ex vivo studies using Atlantic cod (*Gadus morhua*)
2021	Tanja Kofod Petersen	PhD Biology	Biodiversity dynamics in urban areas under changing land-uses
2021	Katariina Vuorinen	PhD Biology	When do ungulates override the climate? Defining the interplay of two key drivers of northern vegetation dynamics
2021	Archana Golla	PhD Biology	Impact of early life stress on behaviour and dorsal raphe serotonergic activity in zebrafish (*Danio rerio*)
2021	Aksel Alstad Mogstad	PhD Biology	Underwater Hyperspectral Imaging as a Tool for Benthic Habitat Mapping

2021	Randi Grønnstad	PhD Biology	Per- and polyfluoroalkyl substances (PFAS) in ski products: Environmental contamination, bioaccumulation and effects in rodents
2021	Gaspard Philis	PhD Biology	Life cycle assessment of sea lice treatments in Norwegian net pens with emphasis on the environmental tradeoffs of salmon aquaculture production systems
2021	Christoffer Høyvik Hilde	PhD Biology	Demographic buffering of vital rates in age-structured populations”
2021	Halldis Ringvold	Dr.Philos	Studies on Echinodermata from the NE Atlantic Ocean - Spatial distribution and abundance of Asteroidea, including taxonomic and molecular studies on Crossaster and Henricia genera- Value-chain results, including test fishery, biology, market and nutritional analysis, on Parastichopus tremulus (Holothuroidea) from the Norwegian coast
2021	Elise Skottene	PhD Biology	Lipid metabolism and diapause timing in Calanus copepods. The impact of predation risk, food availability and oil exposure
2021	Michael Le Pepke	PhD Biology	The ecological and evolutionary role of telomere length in house sparrows
2022	Niklas Erik Johansson	Dr. Philos	On the taxonomy of Northern European Darwin wasps (Hymenoptera: Ichneumonidae).
2022	Jonatan Fredricson Marquez	PhD Biology	Understanding spatial and interspecific processes affecting population dynamics in a marine ecosystem.
2022	Anne Mehlhoop	PhD Biology	Evaluating mitigation measures to reduce negative impacts of infrastructure construction on vegetation and wildlife.
2022	Malene Østreng Nygård	PhD Biology	Integrative biosystematics and conservation genomics – holistic studies of two red-listed plants in Norway
2022	Martin René Ellegaard	PhD Biology	Human Population Genomics in Northern Europe in the Past 2000 years
2022	Gaute Kjærstad	PhD Biology	The eradication of invasive species using rotenone and its impact on freshwater macroinvertebrates
2022	Stefan Vriend	PhD Biology	On the roles of density dependence and environmental fluctuations in driving eco-evolutionary dynamics of hole-nesting passerines
2022	Zaw Min Thant	PhD Biology	Anthropogenic and Environmental factors driving the Human-Elephant Conflict in Myanmar
2022	Prashanna Guragain	PhD Biology	Population analysis and structure and RNA interference to understand salmon lice biology and a review of the principles of controlling infestation in aquaculture facilities.
2022	Ronja Wedegärtner	PhD Biology	Highways up the mountains? Trails as facilitators for redistribution of plant species in mountain areas

2022	Wouter Koch	PhD Biology	Improving the citizen science data corpus for science and management
2022	Qianwen Ding	PhD Biology	Risk of Feed Additives in High-lipid Fish Diets: Effects of Propionate and Docosahexaenoic Acid on Liver and Intestinal Health in Zebrafish (Danio rerio Hamilton-Buchanan, 1822)
2022	Mingxu Xie	PhD Biology	Dietary supplementation of commensal Cetobacterium somerae ameliorates the problems associated with fish meal replacement by plant proteins in fish
2022	Sarah Louise Lundregan	PhD Biology	Impact of genetics and epigenetic processes on parasite infection dynamics in the house sparrow
2022	Stanslaus Mwampeta	PhD Biology	An assessment of field techniques to estimate lion presence and abundance
2023	Mari Aas Fjelldal	PhD Biology	Physiological and ecological challenges faced by small bats in summer
2023	Caitlin Mandeville	PhD Biology	Applications of participatory monitoring in biodiversity science and conservation
2023	Rune Sørås	PhD Biology	Energy management of heterothermic bats at northern latitudes: Understanding the physiological flexibility of bats and how this enables them to live in the northern edge of their distribution
2023	Dilan Saatoglu	PhD Biology	Spatio-temporal dispersal dynamics of a natural house sparrow metapopulation
2023	Christine Klykken	PhD Biology	Nephrocalcinosis in juvenile farmed Atlantic salmon
2023	Anna Haukedal Andreassen	PhD Biology	Mechanisms and evolution of thermal tolerance in fish
2023	Mathew Avarachen	PhD Biology	Carbon sequestration by microbial carbon pump with production of recalcitrant dissolved organic matter in different marine environments
2023	Lisa Sandal	PhD Biology	Spatiotemporal dynamics of avian communities
2023	Ellen Claire Martin	PhD Biology	The role of life history and movement in spatial population synchrony: Theoretical and empirical investigations
2023	Lasse Frost Eriksen	PhD Biology	Life history traits and demography of a mountain bird under climatic variation

ISBN 978-82-326-7804-4 (printed ver.)
ISBN 978-82-326-7803-7 (electronic ver.)
ISSN 1503-8181 (printed ver.)
ISSN 2703-8084 (online ver.)

NTNU
Norwegian University of
Science and Technology