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

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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

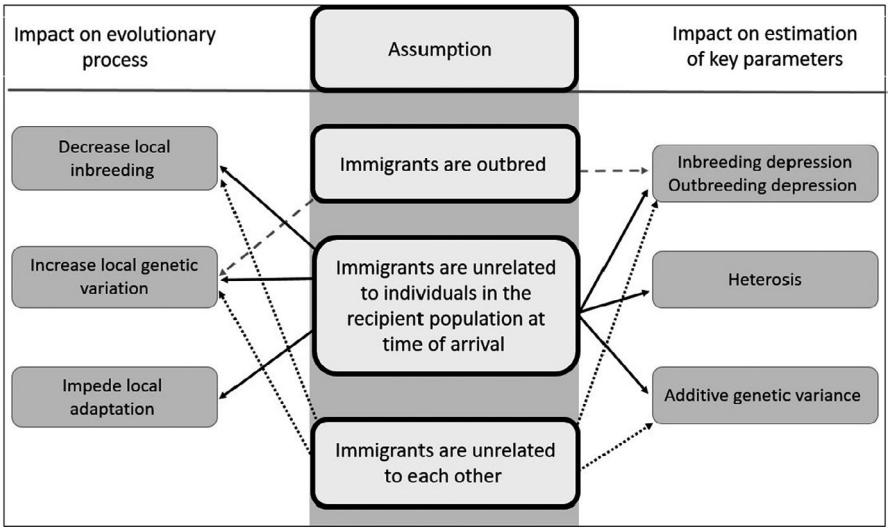

FIGURE 1 Potential main effects of violations of these assumptions on evolutionary effects of immigrants on the recipient population (left column) and on estimation of key parameters (right column). Dashed, solid and dotted lines of connecting arrows indicate the primary links involving the first, second 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 pre-existing 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

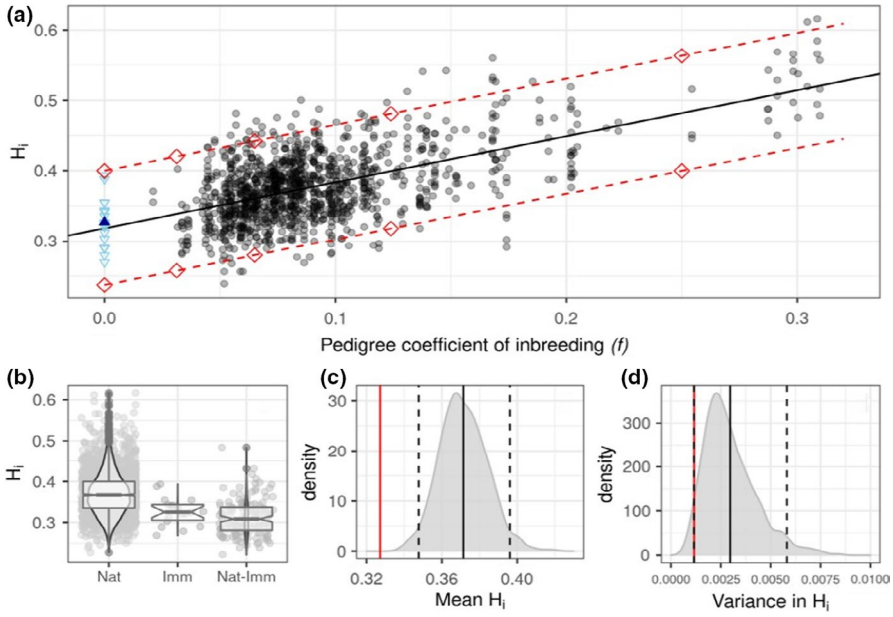

FIGURE 2 Summary of homozygosity of immigrants and natives. (a) Regression of individual homozygosity (H_i) on pedigree coefficient of inbreeding *(f)* across 1,180 defined natives (black line and points; adjusted $R^2 = 0.35$). Light blue hollow triangles indicate 18 immigrants, given the standard assumed 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.

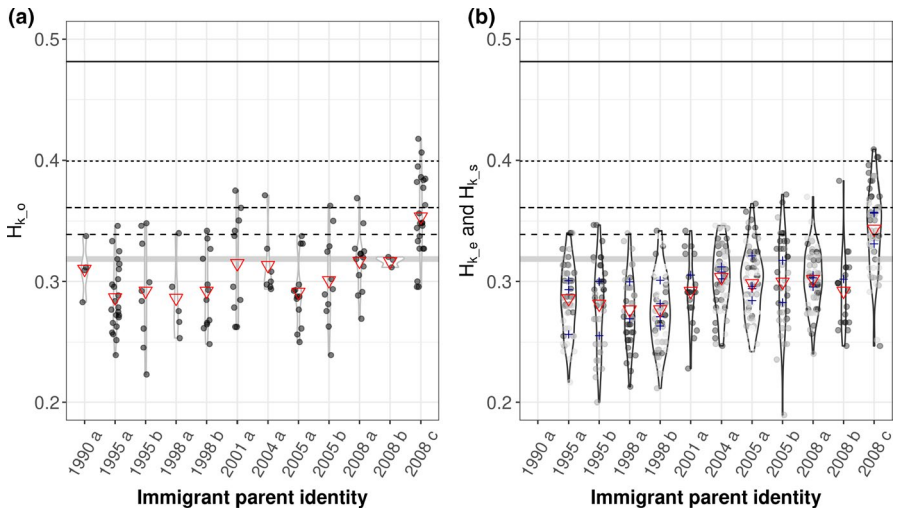

FIGURE 3 Summary of homozygosity of immigrants' offspring. (a) Observed homozygosity of observed offspring of each immigrant (H_{k_o}, grey points), ordered by the immigrant's arrival year with an individual identifier (a,b,c). Red triangles indicate mean H_{k_o} across each immigrant's observed offspring, 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]

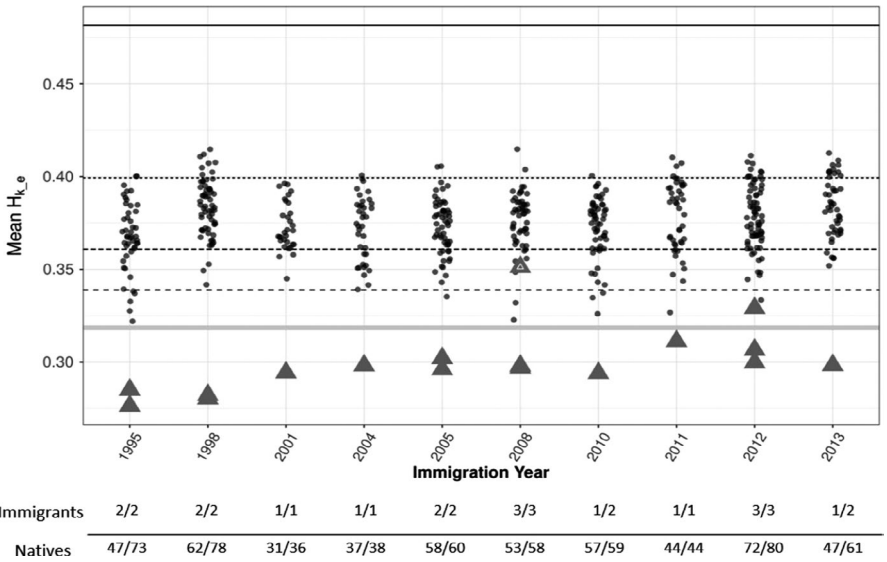

FIGURE 4 Mean expected homozygosity (H_{k_e}) across all possible hypothetical offspring that could be produced by each genotyped immigrant in its year of arrival (triangles) and by each native alive in the same year (points). The immigrant 2008c is indicated with an additional white triangle inside 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

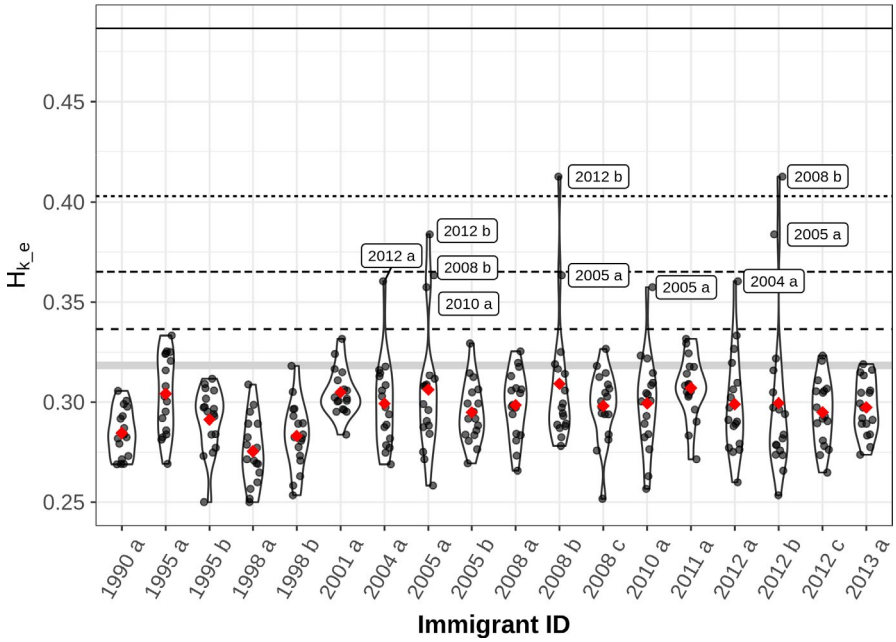

FIGURE 5 Expected homozygosity (H_{k_e}) of hypothetical offspring of immigrant-immigrant pairings. Grey points display H_{k_e} for each focal immigrant in hypothetical pairings with all other immigrants, and violins represent the full distributions. Points are horizontally jittered to aid visibility. 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.

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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).

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