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

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3

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18 Running title: Immigrants and inbreeding

## 19 **Abstract**

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

41 **Key words:** conservation genetics, gene flow, homozygosity, migration, pedigree inbreeding,  
42 relatedness

## 43 **1. Introduction**

44 Immigration and resulting gene flow can substantially influence key evolutionary processes  
45 and consequent persistence of recipient populations (Garant, Forde, & Hendry, 2007).  
46 Specifically, immigration can decrease inbreeding and increase local genetic variation, and  
47 may thereby increase population viability by alleviating expression of inbreeding depression  
48 and generating heterosis (leading to ‘genetic rescue’, reviewed by Frankham, 1998; Tallmon,  
49 Luikart, & Waples, 2004) and/or by facilitating rapid adaptive evolution (leading to  
50 ‘evolutionary rescue’, reviewed by Bell et al., 2019; Carlson, Cunningham, & Westley, 2014;  
51 Gomulkiewicz & Shaw, 2013). Yet, at the same time, immigration could potentially disrupt  
52 the progress of local adaptation, and thereby decrease population viability by constraining  
53 local populations away from their potential fitness peak (i.e. migration load, Bolnick & Nosil,  
54 2007; Garant et al., 2007; Lenormand, 2002). Understanding the diverse genetic impacts of  
55 immigration is therefore central to understanding evolutionary dynamics in metapopulation  
56 systems (Carlson, Cunningham, & Westley, 2014; Garant et al., 2007; Lenormand, 2002;  
57 Reid et al., 2021), predicting fates of populations experiencing changing environments  
58 (Aitken & Whitlock, 2013), conservation of small inbred populations (Frankham, 2015), and  
59 optimal animal breeding (Fernández et al., 2012; Rudnick & Lacy, 2008)

60       Theoretical and empirical studies in all these research areas commonly make three  
61 standard assumptions regarding genetic properties of new immigrants into any focal  
62 population; that such immigrants are 1) outbred, 2) unrelated to all individuals in the existing  
63 recipient population at the time of arrival, and 3) unrelated to each other (Ballou, 1983;

64 Hammerly, de la Cerda, Bailey, & Johnson, 2016; Ivy, Miller, Lacy, & Dewoody, 2009;  
65 Pemberton, 2008; Reid, Arcese, & Keller, 2006; Rudnick & Lacy, 2008; Slate et al., 2004;  
66 Wolak, Arcese, Keller, Nietlisbach, & Reid, 2018). These assumptions, which are often  
67 implicit rather than explicitly stated, underpin general predictions of the degree to which  
68 immigration will decrease inbreeding, cause heterosis, increase local genetic variation and  
69 impede local adaptation (Fig. 1). This in turn implies that violations of any or all of the three  
70 assumptions could mean that immigration will not have the expected effects, and hence that  
71 evolutionary and population dynamic outcomes could differ from standard predictions. For  
72 example, inbred immigrants might directly experience inbreeding depression and/or be less  
73 beneficial for genetic rescue (Frankham, 2015; Ralls, Sunnucks, Lacy, & Frankham, 2020, but  
74 see Heber et al., 2013), while immigrants that are inter-related will likely cause less  
75 outbreeding and weaker heterosis than otherwise expected (Edmands, 2007; Frankham et al.,  
76 2011). Such immigrants will also import fewer novel genetic variants and thus have less  
77 impact on local adaptation and evolution. Furthermore, violations will cause errors in  
78 pedigree-based estimates of coefficients of inbreeding and kinship among immigrants, natives  
79 and their collective descendants. Resulting empirical estimates of key effects, including  
80 inbreeding depression, outbreeding depression, heterosis and additive genetic variance, might  
81 then be biased (Fig. 1). However, despite their foundational role in theoretical and empirical  
82 evolutionary ecology, and potential impacts on conservation and breeding programs  
83 (Fernández et al., 2012; Hammerly et al., 2016), the standard assumptions that new  
84 immigrants are outbred and unrelated to existing natives and to each other are rarely explicitly  
85 tested (Ivy et al., 2009; Robinson et al., 2019). This includes field studies that are central to  
86 empirical understanding of effects of inbreeding and micro-evolution in nature (Marr, Keller,

87 & Arcese, 2002; Reid et al., 2006; Szulkin, Garant, McCleery, & Sheldon, 2007; Wolak et al.,  
88 2018; reviewed by Whiteley, Fitzpatrick, Funk, & Tallmon, 2015).

89         While the assumptions that immigrants are outbred and unrelated may be reasonable  
90 when immigrants originate from large panmictic populations, they might be regularly violated  
91 in natural metapopulations where small sub-populations are connected by dispersal. Here,  
92 immigrants into any focal sub-population might originate from other small sub-populations,  
93 and consequently be as inbred, or even more inbred, than individuals in the focal recipient  
94 sub-population (Chen, Cosgrove, Bowman, Fitzpatrick, & Clark, 2016). Immigrants could  
95 also be related to existing focal sub-population members if there are repeated reciprocal  
96 dispersal events between locations, such that offspring or subsequent descendants of recent  
97 emigrants disperse back into their ancestors' original sub-population. This pattern could be  
98 fueled if dispersal is heritable, increasing the probability that dispersers' offspring will also  
99 disperse (e.g. Doligez & Pärt, 2008). Immigrants might also be related to each other if  
100 individuals disperse alongside relatives and/or in other correlated ways, thereby potentially  
101 impacting genetic variation (Whitlock & McCauley, 1990). Indeed, sibling resemblance in  
102 aspects of dispersal has been observed in birds including great tits (*Parus major*, Matthysen,  
103 Van De Castele, & Adriaensen, 2005), long-tailed tits (*Aegithalos caudatus*, Sharp, Simeoni,  
104 & Hatchwell, 2008), ortolan buntings (*Emberiza hortulana*, Dale, 2010), and house sparrows  
105 (*Passer domesticus*, Billing et al., 2012). Phenotype-dependent, and therefore genotype-  
106 dependent, habitat search and/or settlement could also result in clusters of related dispersers  
107 settling at the same location (e.g. in the warbler-finches *Certhidea olivacea* and *C. fusca*,  
108 Tonnis, Grant, Grant, & Petren, 2005). Dispersal, and resulting immigration, might then be

109 less random than is commonly assumed (Doligez & Pärt, 2008; Edelaar & Bolnick, 2012).  
110 Explicitly quantifying the degree to which new immigrants are in fact outbred and unrelated  
111 to natives and to each other, and adjusting subsequent analyses accordingly, could ultimately  
112 allow more precise and unbiased estimates of effects of inbreeding and outbreeding, and  
113 thereby help resolve ongoing debates regarding the various genetic effects of immigrants  
114 (Gomulkiewicz & Shaw, 2013; Reid et al., 2021). Such quantification could also contribute to  
115 general understanding of the degree of non-random immigration and the circumstances under  
116 which it occurs.

117         Testing the three standard assumptions (Fig. 1) requires estimation of individual  
118 coefficient of inbreeding ( $f$ ) and pairwise coefficients of kinship ( $k$ ) in and among new  
119 immigrants and pre-existing natives on a common quantitative scale.  $K$  between two  
120 individuals equals  $f$  of resulting offspring, meaning that  $k$  can be directly inferred from  
121 offspring  $f$  and vice versa (Falconer & Mackay, 1996, Supplemental Information B). The  
122 coefficients  $f$  and  $k$  have traditionally been calculated from population pedigree data, and this  
123 remains a valuable approach (Galla et al., 2020; Nietlisbach et al., 2017; Pemberton, 2008).  
124 Expected values of  $f$  and  $k$  are calculated relative to a defined pedigree baseline ‘founder’  
125 population, typically taken as the set of individuals alive at the start of the study or breeding  
126 program (i.e. with unknown parents, Hogg et al., 2019; Lacy, 1989). Since subsequent  
127 immigrants are by definition not born within the focal population, their parents, grandparents  
128 and more distant ancestors are usually unknown. Standard pedigree analyses then include  
129 such immigrants in the defined base population, thereby making the standard assumptions that  
130 they are outbred and unrelated, thereby precluding direct estimation of  $k$  and  $f$  for the



131 immigrants and potentially introducing influential pedigree errors if the standard assumptions  
132 are violated (Ivy et al., 2009; Rudnick & Lacy, 2008; Wolak & Reid 2017).

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

151         Long-term data from a song sparrow (*Melospiza melodia*) population resident on  
152 Mandarte Island, British Columbia, Canada, have proved valuable for examining the

153 occurrence and consequences of inbreeding in the wild (Keller, 1998; Keller & Arcese 1998;  
154 Reid et al., 2014, 2015, 2016), and the quantitative genetic basis of life-history variation  
155 (Reid, Arcese, Sardell, & Keller, 2011; Reid & Sardell, 2012; Wolak et al., 2018), including  
156 consequences of immigration (Marr et al., 2002; Reid & Arcese, 2020; Reid et al., 2021;  
157 Wolak et al., 2018). Recent analyses utilized complete multi-year pedigree data, where  
158 genetic parentage of focal Mandarte-hatched individuals was verified with very high  
159 statistical confidence (Nietlisbach et al., 2017; Reid et al., 2011, 2014, 2021; Sardell, Keller,  
160 Arcese, Bucher, & Reid, 2010). However, as with all such studies, pedigree analyses invoked  
161 the three standard assumptions that new immigrants are outbred, unrelated to the native  
162 population at the time of arrival and unrelated to each other; but these assumptions have not  
163 been explicitly validated. Accordingly, we used genotypic data from 150-160 polymorphic  
164 microsatellite loci to estimate marker homozygosity and infer the degrees of inbreeding and  
165 kinship of immigrant song sparrows, for which ancestral pedigree data are unavailable, in  
166 relation to the multi-generational pedigree baseline for existing natives. We thereby evaluate  
167 to what degree the three standard assumptions hold, highlight how they could be tested in  
168 other systems, and consider the implications for estimates of key evolutionary processes and  
169 outcomes.

## 170 **2. Materials and Methods**

### 171 **Study system**

172 Mandarte's song sparrow population has been studied intensively since 1975. In brief, all  
173 territories and nests were closely monitored, and all chicks reaching  $\geq 6$  days old were marked  
174 with unique combinations of colored plastic and metal bands. All individuals that locally  
175 recruit as adults (age 1 year) are consequently individually identifiable (Arcese, Smith, &  
176 Hochachka, 1992; Keller, 1998; Marr et al., 2002; Smith, Keller, Marr, & Arcese, 2006;  
177 Wolak et al., 2018). The occasional immigrants to Mandarte are initially recognizable as  
178 unbanded adults present in spring, and are subsequently mist-netted and individually color-  
179 banded. In total, 48 immigrants arrived during 1976-2016 (30 females, 18 males), with a  
180 mean of 1.2/year (range 0-4) with arrivals in 28 different years.

181         Since the total local population size is small (mean  $77.2 \pm 38.3$  SD adults/year, range  
182 12-159) and most immigrants successfully reproduced, the combined immigrants made a  
183 substantial genetic contribution to subsequent generations (Keller et al., 2001; Reid & Arcese,  
184 2020; Reid et al., 2021; Wolak et al., 2018). Since Mandarte (latitude  $48.6329^\circ$ , longitude -  
185  $123.2859^\circ$ ,  $0.06 \text{ km}^2$ ) lies within  $\leq 5$  km of several other small islands (and 8 km away from  
186 Vancouver Island,  $31,300 \text{ km}^2$ ), immigrants could potentially be inbred and/or related. The  
187 three standard assumptions (Fig. 1) should consequently be explicitly verified.

188

### 189 **Genotypic data and measures of inbreeding and relatedness**

190 All sparrows alive on Mandarte during 1993-2013 ( $n=3644$ ) were blood-sampled and initially  
191 genotyped at 13 highly polymorphic microsatellite markers to allow assignment of genetic  
192 parentage and compilation of a complete and accurate pedigree (all parents assigned with >99  
193 % individual-level confidence, Nietlisbach et al., 2017; Reid et al., 2014; Sardell et al., 2010;  
194 Wolak et al., 2018). This sample includes individuals that hatched on or immigrated to  
195 Mandarte during 1993-2013, alongside some surviving individuals that hatched or arrived in  
196 earlier years. Previous analyses of resulting pedigree data showed that mean  $f$  varied little  
197 across years (Reid et al, 2021), with no evidence of non-random mating with respect to  
198 kinship (i.e. inbreeding preference or avoidance, Keller and Arcese, 1998; Reid et al., 2015).  
199 The genetic marker data also verified the status of all presumed immigrants, since all adults  
200 alive in the breeding season before each immigrant's apparent arrival were excluded as their  
201 genetic parents with high confidence.

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

209 Several moment and maximum likelihood methods to estimate inbreeding and  
210 relatedness (or kinship) from genetic marker (e.g. microsatellite) data have been derived  
211 (Wang, 2014). However, such estimators are problematic to interpret when sampled

212 individuals come from heterogeneous source populations, as with mixtures of immigrants and  
213 natives (Nietlisbach et al., 2018). This is partly because many such estimators use estimates of  
214 allele frequencies to attempt to distinguish homozygosity due to recent inbreeding and  
215 resulting identity by descent from identity by state (summarized in Keller, Visscher, &  
216 Goddard, 2011; Slate et al., 2004; Wang, 2014). These allele frequencies can be estimated  
217 from available samples from single focal populations, but may differ for immigrants arriving  
218 from other unobserved populations (Fienieg & Galbusera, 2013; Wang, 2014). Performance  
219 and interpretation of such estimators can therefore depend on actual relatedness and  
220 metapopulation structure and resulting allelic frequency variation (e.g. Blouin, 2003;  
221 Oliehoek, Windig, Van Arendonk, & Bijma, 2006; Wang, 2011). Estimators can also be  
222 biased if there are numerous inbred or closely related individuals within the reference  
223 population (Wang, 2014), or if there is a high proportion of related individuals alongside  
224 unrelated individuals in the examined sample (Goudet & Weir 2018; Csilléry, et al. 2006).  
225 These conditions apply on *Mandarte*, where there is substantial inbreeding (Germain, Arcese,  
226 & Reid, 2018; Nietlisbach et al., 2017; Reid et al., 2014, 2015, 2016) and the origins of recent  
227 immigrants and associated sub-population allele frequencies are unknown. Such estimators  
228 are consequently not appropriate for our current analyses.

229         Instead, basic estimates of inbreeding and kinship between individuals that do not  
230 explicitly incorporate allele frequencies can be obtained simply by computing marker  
231 homozygosity for observed immigrants and natives, and for their real or hypothetical  
232 offspring. Hence, to achieve our current objectives, we estimated each individual's degree of  
233 inbreeding as the proportion of genotyped microsatellite loci that were homozygous (i.e.

234 number of homozygous loci divided by total genotyped loci, hereafter  $H_i$ ). We estimated  
235 kinship between any two focal individuals as the proportion of loci that were observed to be  
236 homozygous in real offspring of observed pairings, or expected to be homozygous in potential  
237 offspring of hypothetical pairings (hereafter  $H_k$ ; further explanations below). This relies on  
238 the point that  $k$  between two individuals equals  $f$  of their offspring (Supplemental Information  
239 B). We did not standardize estimates by expected homozygosity at each locus (i.e. accounting  
240 for allele frequencies) because the expectation is unknown for immigrants, and previous  
241 analyses showed that unstandardized and standardized measures of homozygosity were highly  
242 correlated across non-immigrant individuals (correlation coefficient  $r=0.999$ , Nietlisbach et  
243 al., 2017). A linkage map showed that the focal microsatellite loci are widely distributed  
244 across the genome (Nietlisbach et al., 2015), implying that observed marker homozygosity  
245 will broadly represent genome-wide homozygosity.

246         Alongside the advantages, there are also some challenges of using marker  
247 homozygosity to estimate inbreeding and kinship, which our analyses were designed to  
248 ameliorate. Resulting values of  $H_i$  and  $H_k$  presumably represent some degree of identity by  
249 state alongside identity by descent, and therefore do not quantitatively equal the pedigree-  
250 derived metrics  $f$  and  $k$  (Slate et al., 2004; Falconer & Mackay, 1996). Hence, to facilitate  
251 interpretation, we benchmarked values of  $H_i$  (and hence  $H_k$ ) against pedigree  $f$ . We extracted  
252 values of  $f$  for genotyped ‘natives’ (defined here as individuals whose parents and  
253 grandparents hatched on Mandarte and hence whose recent ancestors were not immigrants)  
254 calculated from the full Mandarte pedigree, and regressed  $H_i$  on  $f$  across these individuals. To  
255 estimate a value of  $H_i$  that broadly represents  $f=0$  relative to the defined pedigree baseline, we  
256 extracted the regression intercept with its 95% prediction interval. This intercept indicates

257 what value of  $H_i$  implies that a song sparrow is ‘outbred’ on the scale defined by the  
258 pedigreed Mandarte population. The prediction interval indicates the range of  $H_i$  values that  
259 could plausibly be observed in individuals with pedigree  $f=0$ . We also extracted mean  $H_i$  for  
260 four further biologically meaningful values of  $f$ , corresponding to offspring of first-degree  
261 relatives (full-sibling or parent-offspring pairings,  $f=0.25$ ), second-degree relatives (e.g. half-  
262 siblings,  $f=0.125$ ), third-degree relatives (e.g. first cousins,  $f=0.0625$ ), and fourth-degree  
263 relatives (e.g. an individual with its first cousin once removed,  $f=0.03125$ ). We did not  
264 directly extract  $H_i$  for individuals with pedigree  $f=0$  because the only genotyped individuals  
265 with  $f=0$  are immigrants and their offspring. These individuals are assigned  $f=0$  due to the  
266 three standard assumptions (i.e. that immigrants are outbred and unrelated), which is what we  
267 currently aim to test. Directly benchmarking  $H_i$  against  $f$  using these individuals would  
268 consequently be meaningless.

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

280 by estimators that treat inbreeding as a correlation coefficient rather than a probability of  
281 identity by descent (Wang, 2014).

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

292

### 293 **Testing assumption 1: Are immigrants outbred?**

294 To test the assumption that immigrants were outbred relative to the native base population, we  
295 first calculated  $H_i$  for 18 immigrants that were alive on Mandarte at some point during 1993-  
296 2013 and hence were genotyped at 150-159 microsatellite loci (mean  $155.10 \pm 2.59$  SD). These  
297 immigrants arrived in eleven different years during 1990-2013. We then calculated  $H_i$  for  
298 1908 defined natives from the same period that were genotyped at 150-160 loci (mean  
299  $157.00 \pm 2.82$  SD; Supplemental Information A). We used a Kolmogorov-Smirnov test to  
300 examine whether  $H_i$  for immigrants and natives likely derived from the same distribution  
301 defined by shape and location. Due to highly unbalanced sample sizes, we also directly tested



302 whether the observed distribution of immigrant  $H_i$  differed from that which could be drawn by  
303 chance given the observed distribution of native  $H_i$ . Specifically, we randomly drew 18  
304 observations of  $H_i$  from all natives, calculated the sample mean and variance across 1000  
305 iterations, and examined whether the observed mean and variance of the immigrants'  $H_i$  fell  
306 within the central 95% confidence interval of the simulated range. We additionally compared  
307 mean  $H_i$  estimated across the 18 immigrants to the benchmark for pedigree  $f=0$ , and examined  
308 whether individual  $H_i$  values fell within the 95% prediction interval.

309

### 310 **Testing assumption 2: Are immigrants unrelated to natives?**

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

319         However, such analyses of real observed offspring obviously incompletely describe  
320 the kinship between new immigrants and all existing natives. Not all immigrants reproduced,  
321 or reproduced during the years in which offspring were genotyped at  $\geq 150$  loci. Of course, no  
322 immigrants reproduced with all opposite-sex natives, or with any same-sex natives.  
323 Immigrants might therefore have close relatives in the existing population that would not be

324 detected through analyses of real offspring. Further, as noted above, since substantial  
325 Mendelian sampling variance in estimates of  $H_{k_o}$  should be expected, observation of a single  
326 real offspring with relatively high  $H_{k_o}$  does not necessarily mean that its immigrant and  
327 native parents were particularly closely related.

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

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

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

344

345 **Testing assumption 3: Are immigrants unrelated to each other?**

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

354

## 355 **Implementation**

356 All analyses were implemented using R version 3.6.3 (R core Team, 2020), using the  
357 tidyverse framework for data exploration, summary and visualization (Wickham et al., 2019)  
358 and package nadiv (Wolak, 2012) for pedigree analysis. All field data collection was  
359 approved by the University of British Columbia Animal Care Committee and conducted  
360 under banding permits from Environment and Climate Change Canada and Government of  
361 British Columbia. Data will be available from Dryad following manuscript acceptance  
362 (Dickel et al., 2021).

### 363 **3. Results**

#### 364 **Benchmarking, and homozygosity of immigrants versus natives**

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

371 Mean  $H_i$  was  $0.37 \pm 0.05$  SD (range  $0.23-0.62$ ) across the 1908 genotyped natives (and  
372 was quantitatively similar across 443 natives that survived to adulthood). Meanwhile, mean  $H_i$   
373 was  $0.33 \pm 0.03$  SD (range  $0.27-0.39$ ) across the 18 genotyped immigrants (Fig. 2B). The  
374 distribution of  $H_i$  differed between the two groups (two sample Kolmogorov-Smirnov test,  
375  $D=0.48$ ,  $p<0.01$ ). Additional simulations confirmed that both the mean and the variance of  $H_i$   
376 were smaller in the 18 observed immigrants than in 18 randomly drawn natives (Fig. 2C, 2D).

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

382

#### 383 **Relatedness of immigrants to natives**

384 There was a total of 133 genotyped real offspring of immigrant-native pairings, produced by  
385 12 of the 18 genotyped immigrants. Mean  $H_{k_o}$  across these offspring was  $0.31 \pm 0.04$  SD  
386 (range 0.22-0.42, Fig. 3A), corresponding closely to the benchmark of  $H_i = 0.32$  for pedigree  
387  $f = 0$ . Most immigrants' offspring were less homozygous than the genotyped natives, and than  
388 the immigrants themselves (Fig. 2B). This implies that reproducing immigrants were typically  
389 unrelated to their native mates. However, one immigrant produced relatively homozygous  
390 offspring, broadly comparable to the estimated value for offspring of third-degree relatives  
391 (Fig. 3A, individual 2008c). This implies that this immigrant was distantly related to its native  
392 mate. Observed variation in  $H_{k_o}$  (i.e. observed offspring homozygosity) was similar to the  
393 sampling variance evident in  $H_{k_s}$  (i.e. simulated offspring homozygosity), while  $H_{k_e}$  (i.e.  
394 expected offspring homozygosity) was quantitatively similar to mean  $H_{k_o}$  and mean  $H_{k_s}$ .  
395 This demonstrates substantial Mendelian sampling variance in  $H_{k_s}$  and hence  $H_{k_o}$ . Mean  
396  $H_{k_o}$ , and  $H_{k_s}$ , and  $H_{k_e}$  are consequently the preferred values for inference.

397 In total, there were 26,626 possible native-native pairings and 1,850 possible  
398 immigrant-native pairings involving genotyped immigrants and natives alive in the  
399 immigrants' arrival years, representing 10 years between 1995 and 2013. On average,  
400  $88 \pm 11\%$  (range 65%-100%) of adults alive in each year were genotyped at 150-160 loci (Fig.  
401 4). It is therefore very unlikely that numerous natives to which an immigrant was related were  
402 excluded from analyses. Across all possible pairings, mean  $H_{k_e}$  was  $0.38 \pm 0.45$  SD (range  
403 0.27-0.65) for hypothetical offspring of native-native pairings and  $0.30 \pm 0.02$  SD (range 0.24-  
404 0.40) for hypothetical offspring of immigrant-native pairings. Mean  $H_{k_e}$  for the hypothetical  
405 offspring of each immigrant was typically considerably lower than mean  $H_{k_e}$  for the  
406 hypothetical offspring of natives alive in the same year (Fig. 4). Mean  $H_{k_e}$  for most

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

416 Immigrants imported on average  $36.2 \pm 8.6$  SD microsatellite alleles per individual that  
417 were not present in the genotyped sample of existing Mandarte adults alive at time of arrival  
418 (range 17-47, Supplemental Information E). This directly indicates that immigrants were not  
419 closely related to the existing population and introduced novel genetic variation.

420

#### 421 **Relatedness of immigrants to each other**

422 Mean  $H_{k_e}$  of hypothetical offspring of all 153 possible immigrant-immigrant pairings was  
423  $0.30 \pm 0.02$  SD (range 0.25–0.41), and hence slightly below the benchmark for pedigree  $f=0$   
424 (Fig. 5). Yet, there was some variation, and two immigrants would have produced offspring of  
425 similar homozygosity to offspring of second-degree relatives on Mandarte (2012b and 2008b,  
426 Fig. 5).

## 427 **Discussion**

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

440

### 441 **Are immigrants outbred?**

442 Pedigree data for immigrants and their ancestors, and knowledge of immigrants' origins, are  
443 rarely available unless field studies encompass entire meta-population systems (e.g. Billing et  
444 al., 2012; Niskanen et al., 2020) or immigration is facilitated by conservation programs (e.g.  
445 Hasselgren et al., 2018). Consequently, pedigree coefficients of inbreeding ( $f$ ) cannot typically  
446 be meaningfully directly calculated for immigrants, which are then either assumed to be  
447 outbred or excluded from analyses (e.g. Keller 1998; Reid et al., 2014; Szulkin et al., 2007;

448 Wolak et al., 2018). Our combined analyses of multi-locus microsatellite homozygosity ( $H_i$ )  
449 and pedigree data showed that immigrants to Mandarte were on average less homozygous  
450 than existing natives, with mean  $H_i$  close to the estimated benchmark for pedigree  $f=0$ , and  
451 individual values that fell within the 95 % prediction interval. The genotyped immigrants can  
452 consequently be interpreted to be effectively outbred relative to the defined Mandarte  
453 population baseline.

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

461         This situation, and the corresponding assumption that immigrants are relatively  
462 outbred, are less likely to be valid when a focal population is not geographically isolated or  
463 particularly small compared to immigrants' source populations. Indeed, there are other small,  
464 inbred song sparrow populations on islands close to Mandarte, from which immigrants could  
465 potentially originate (Marr et al., 2002; Wilson & Arcese, 2008). The observed variation in  $H_i$   
466 among immigrants to Mandarte could consequently reflect arrival of some relatively inbred  
467 individuals. However, the range of variation observed for immigrants does not exceed that  
468 observed for natives given any one value of pedigree  $f$ , or exceed simulated variation in  
469 offspring homozygosity. It could therefore simply reflect Mendelian and/or marker sampling  
470 variance. Future studies with much higher density mapped genomic data will allow tighter



471 direct estimation of inbreeding coefficients of individual immigrants and natives, for example  
472 using runs of homozygosity (ROH). Such methods capture variance due to Mendelian  
473 inheritance and reduce the marker sampling variance, and thereby allow stronger individual-  
474 level rather than solely group-level inferences, and also give insights into histories of  
475 inbreeding events (Goudet et al. 2018; Hedrick & Garcia-Dorado, 2016; Kardos, Luikart, &  
476 Allendorf, 2015; Niskanen et al., 2020; Robinson et al., 2019).

477

### 478 **Are immigrants unrelated?**

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

491 Further, our analyses show that immigrants' offspring would generally be even less  
492 homozygous than the benchmark for pedigree  $f=0$ . Alongside the introduction of new alleles,

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

508         Yet, despite the strong evidence that immigrants are typically unrelated to existing  
509 natives at arrival, one immigrant (arrived in 2008) was apparently somewhat related to the  
510 natives. A second immigrant (arrived in 2012) would also have produced offspring that were  
511 slightly more homozygous than the benchmark for pedigree  $f=0$ . Such non-zero relatedness  
512 between immigrants and natives could potentially result from different dispersal patterns.  
513 First, it could reflect sequential reciprocal dispersal, where an emigrant's descendants disperse  
514 back to their ancestor's source population. Second, it could also arise if there is repeated  
515 directional immigration from the same source population across years. Non-zero relatedness

516 between new arrivals and defined natives could then arise because the natives include  
517 descendants of previous immigrants. This would imply that immigrants that are apparently  
518 related to the native population are also related to at least one other immigrant. However,  
519 there was little evidence of such effects in our current data set. Specifically, there was little  
520 evidence that sampled immigrants were closely related to each other; rather the expected  $H_{k_e}$   
521 of hypothetical immigrant-immigrant offspring was typically below the benchmark for  
522 pedigree  $f=0$ . This implies that the immigrants come from a large source population and/or  
523 from different source populations, with no evidence of non-independent dispersal between  
524 related individuals, as observed in other passerine birds. For example, in house sparrows  
525 (*Passer domesticus*, Billing et al., 2012) and long-tailed tits (*Aegithalos caudatus*, Sharp et al.,  
526 2008) sibling pairs were detected among immigrants, in ortolan buntings (*Emberiza*  
527 *hortulana*) dispersal direction of siblings were similar (Dale, 2010), and in great tits (*Parus*  
528 *major*) siblings bred closer together than expected by chance (Matthysen et al., 2005).

529

### 530 **Implications and applications**

531 The practical relevance (and fitness consequences) of mis-assigned relatedness between  
532 individuals has previously been demonstrated in the context of conservation breeding  
533 programs. For example in Attwater's prairie-chickens (*Tympanuchus cupido attwateri*), mean  
534 relatedness of parents was significantly reduced by using molecular relatedness information to  
535 identify optimal breeding pairs, leading to higher chick survival (Hammerly et al., 2016). In  
536 contrast, a simulation study based on data from captive parma wallabies (*Macropus parma*)  
537 found that molecular genetic estimates of relatedness would have little potential for improving

538 genetic management by matching unrelated pairs for breeding, possibly because there were  
539 few unknown close relatives (Ivy et al., 2009). Indeed, general simulations have shown that,  
540 in the short term, offspring fitness would only be substantially increased when previously  
541 undetected close relatives (e.g. full siblings) are revealed (Rudnick & Lacy 2008). Even here,  
542 impacts decrease over generations, meaning that long-term effects were minor. These insights  
543 from conservation genetics could be taken to imply that only recent inbreeding events are  
544 practically relevant to predicting population outcomes (Rudnick & Lacy, 2008, reviewed in  
545 Fienieg & Galbusera, 2013).

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

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

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841 **Data Accessibility**

842 The data used in this study will be available from the Dryad digital repository if the  
843 manuscript is accepted for publication.

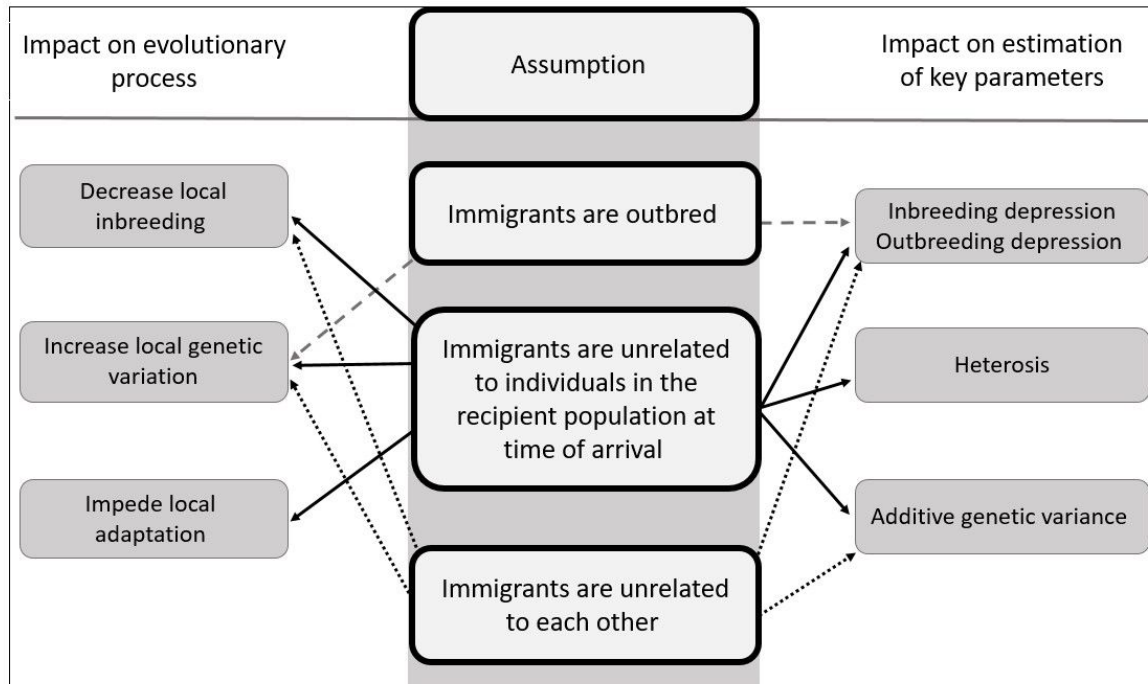
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845 **Author Contributions**

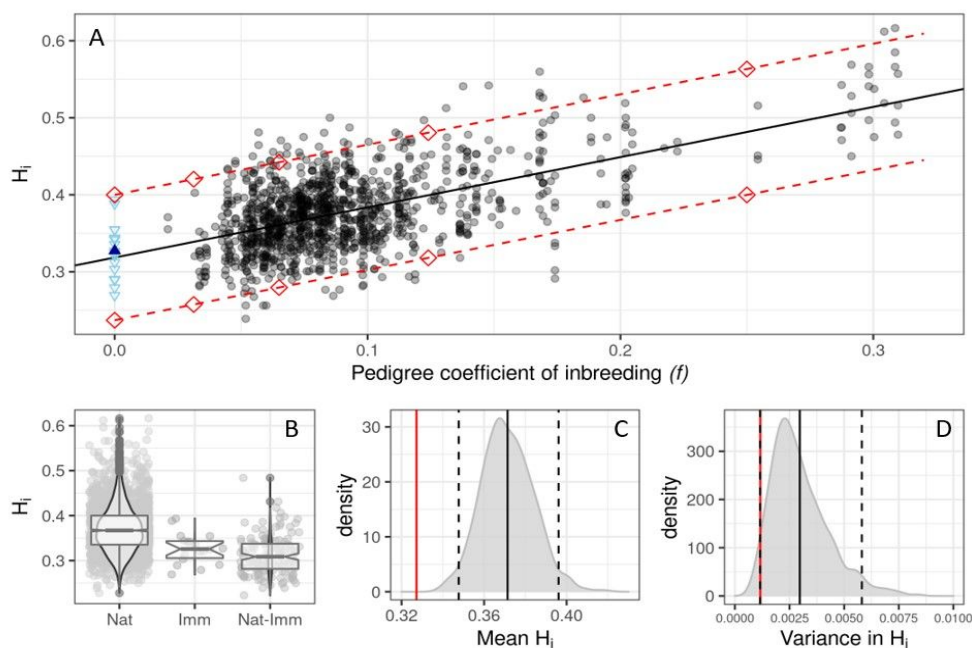
846 L.D. undertook the analyses and drafted the manuscript in collaboration with J.M.R. P.A.  
847 undertook and oversaw long-term field data collection. P.N. and L.F.K. led genotyping and  
848 pedigree reconstruction. P.N., L.F.K and J.M.R. contributed to fieldwork. All authors  
849 contributed substantially to conceptual development and manuscript editing.

850 **Figures and Tables**

851

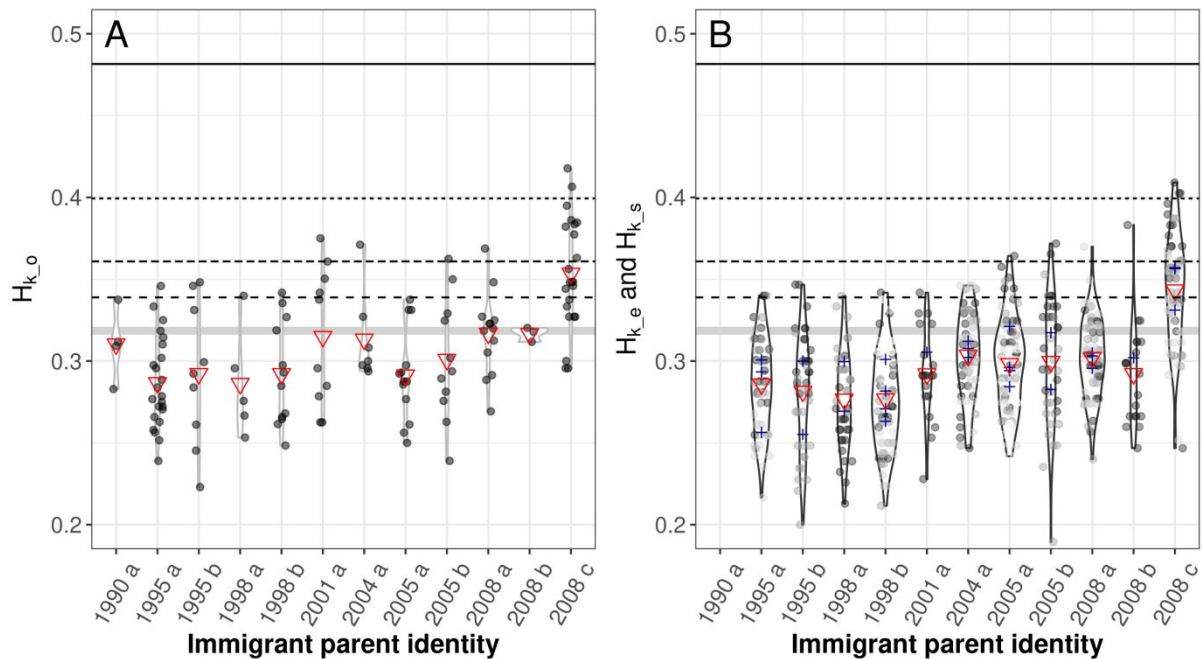


852 **Figure 1:** Potential main effects of violations of these assumptions on evolutionary effects of  
 853 immigrants on the recipient population (left column) and on estimation of key parameters  
 854 (right column). Dashed, solid and dotted lines of connecting arrows indicate the primary links  
 855 involving the first, second and third assumptions respectively.



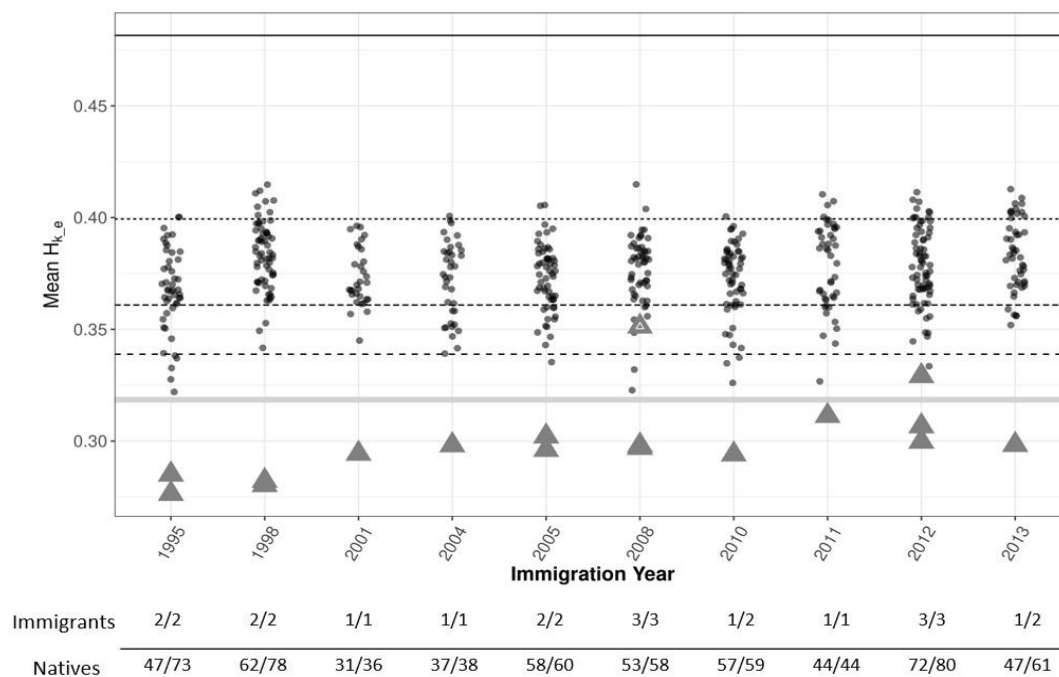
856 **Figure 2:** Summary of homozygosity of immigrants and natives. **A.** Regression of individual  
 857 homozygosity ( $H_i$ ) on pedigree coefficient of inbreeding ( $f$ ) across 1180 defined natives  
 858 (black line and points; adjusted  $R^2=0.35$ ). Light blue hollow triangles indicate 18 immigrants,  
 859 given the standard assumed inbreeding coefficient of  $f=0$ . The dark blue filled triangle  
 860 indicates the immigrants' mean (the median is quantitatively similar). Red diamonds indicate  
 861 prediction intervals for biologically meaningful categories comprising offspring of first-,  
 862 second-, third- and fourth-degree relatives and unrelated individuals ( $f=0$ ), connected by the  
 863 red dotted line for visualization purposes. **B.** Observed  $H_i$  of 1908 natives (Nat), 18  
 864 immigrants (Imm) and 133 immigrant-native offspring (Nat-Imm). Box plots indicate the  
 865 median and quartiles. Violins indicate the full distribution. Points represent individuals, and  
 866 are horizontally jittered to aid visibility. **C and D.** Density distributions of mean (C) and  
 867 variance (D) in  $H_i$  across 1000 random samples of 18 natives. Solid and dashed lines denote  
 868 means and 95% confidence intervals, respectively. Red lines denote the observed mean and  
 869 variance in  $H_i$  across the 18 observed immigrants.





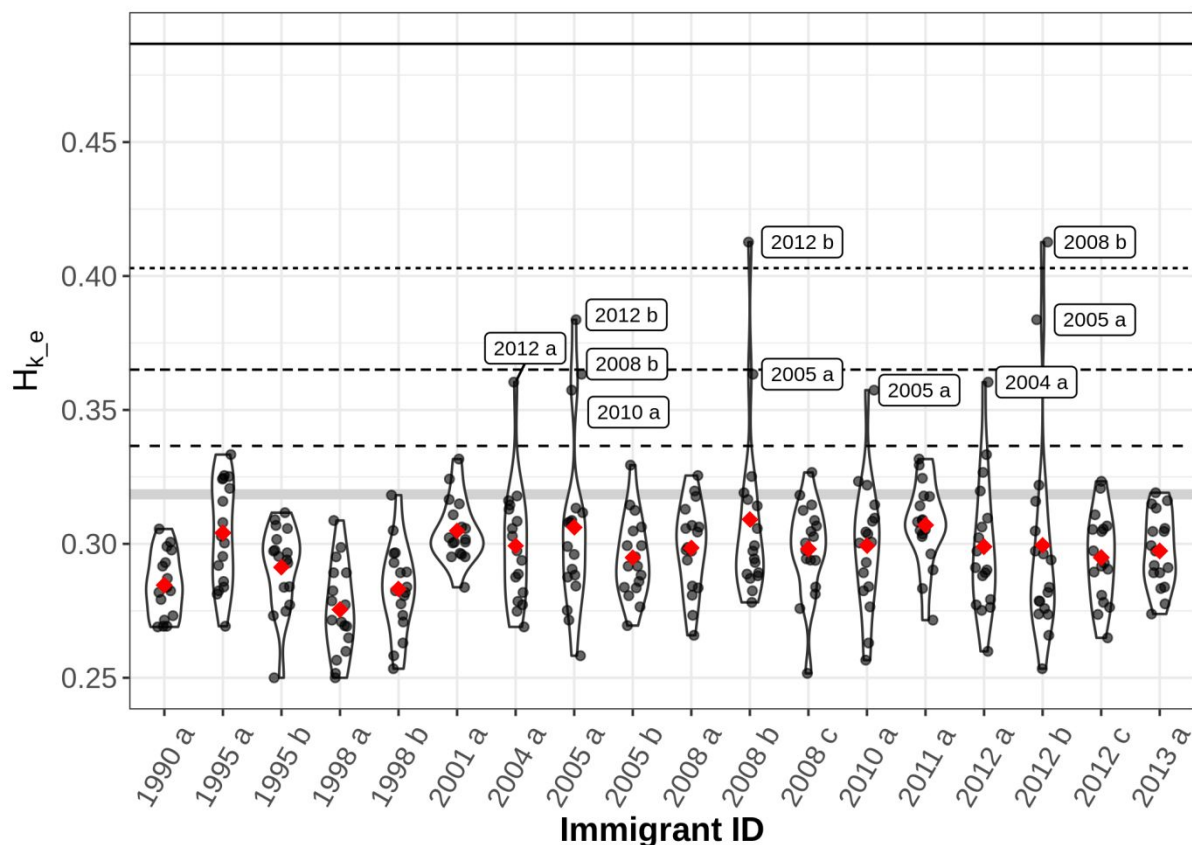
870

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



883

884 **Figure 4:** Mean expected homozygosity ( $H_{k_e}$ ) across all possible hypothetical offspring that  
 885 could be produced by each genotyped immigrant in its year of arrival (triangles) and by each  
 886 native alive in the same year (points). The immigrant 2008c is indicated with an additional  
 887 white triangle inside the gray triangle. Points are horizontally jittered to aid visibility.  
 888 Horizontal lines indicate predicted  $H_i$  benchmarks for values of pedigree  $f$  of 0.25 (solid),  
 889 0.125 (dotted), 0.0625 (dashed) and 0.01325 (spaced dashed). The grey band shows the  
 890 benchmark value of  $H_i$  for pedigree  $f=0$ . Numbers below show the number of immigrants  
 891 ( $Imm$ ) that arrived in each year, and the number of adult natives ( $Nat$ ) alive in each year,  
 892 which were genotyped at  $\geq 150$  loci (left of slash) versus the total number (right of slash) in  
 893 each category. Full underlying distributions of  $H_{k_e}$  are shown in Supplemental Information  
 894 Figure C.



895

896 **Figure 5:** Expected homozygosity ( $H_{k_e}$ ) of hypothetical offspring of immigrant-immigrant  
 897 pairings. Grey points display  $H_{k_e}$  for each focal immigrant in hypothetical pairings with all  
 898 other immigrants, and violins represent the full distributions. Points are horizontally jittered to  
 899 aid visibility. Red diamonds indicate mean  $H_{k_e}$  for each immigrant. Horizontal lines indicate  
 900 predicted  $H_i$  benchmarks for values of pedigree  $f$  of 0.25 (solid), 0.125 (dotted), 0.0625  
 901 (dashed) and 0.01325 (spaced dashed). The grey band shows the benchmark value of  $H_i$  for  
 902 pedigree  $f=0$ . Flags indicate the identity of the second immigrant parent of hypothetical  
 903 offspring, indicating pairings with relatedness resulting in offspring comparable to fourth-  
 904 degree relative offspring or closer.