

Demographic History, Not Mating System, Explains Signatures of Inbreeding and Inbreeding Depression in a Large Outbred Population

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ABSTRACT: Inbreeding depression is often found in small, inbred populations, but whether it can be detected in and have evolutionary consequences for large, wide-ranging populations is poorly known. Here, we investigate the possibility of inbreeding in a large population to determine whether mild levels of inbreeding can still have genetic and phenotypic consequences and how genomically widespread these effects can be. We apply genome-wide methods to investigate whether individual and parental heterozygosity is related to morphological, growth, or life-history traits in a pelagic seabird, Leach's storm-petrel (*Oceanodroma leucorhoa*). Examining 560 individuals as part of a multiyear study, we found a substantial effect of maternal heterozygosity on chick traits: chicks from less heterozygous (relatively inbred) mothers were significantly smaller than chicks from more heterozygous (noninbred) mothers. We show that these heterozygosity–fitness correlations were due to general genome-wide effects and demonstrate a correlation between heterozygosity and inbreeding, suggesting inbreeding depression. We used population genetic models to further show that the variance in inbreeding was probably due to past demographic events rather than the current mating system and ongoing mate choice. Our findings demonstrate that inbreeding depression can be observed in large populations and illustrate how the integration of genomic techniques and fieldwork can elucidate its underlying causes.

Keywords: double-digest restriction site–associated DNA sequencing (ddRAD-seq), extra-pair paternity, heterozygosity–fitness correlation, multilocus heterozygosity, *Oceanodroma leucorhoa*, relatedness.

Introduction

Inbreeding depression (the association between inbreeding and a reduction in fitness-related traits) is of major interest in ecology, evolution, and conservation. Inbreeding (mating between relatives) increases the probability that two homologous alleles will be descended from a recent common ancestor (i.e., identical by descent [IBD]), which reduces the genome-wide heterozygosity of offspring. Inbreeding reduces fitness by exposing recessive or partially recessive deleterious alleles or by decreasing heterozygote advantage (Charlesworth and Charlesworth 1987; Keller and Waller 2002), both of which lead to negative effects on the fitness of inbred compared with outbred individuals. At the population level, variation in inbreeding generates patterns of genome-wide genotypic variation among individuals (identity disequilibrium), which in turn drives fitness variation among individuals (inbreeding depression). Inbreeding has therefore been proposed to generate heterozygosity–fitness correlations (HFCs) at the population level—a statistical association between genome-wide heterozygosity and fitness. Although HFCs can arise from inbreeding (called “general effects”), they can also be produced by genomic “local effects”: linkage disequilibrium between markers under study and nearby genes or genomic regions with strong fitness effects (Hansson and Westerberg 2002). The general effects interpretation of HFCs implies that interindividual variation in multilocus heterozygosity captures identity disequilibrium when associations between heterozygosity and fitness at the population level reflect inbreeding depression (Szulkin et al. 2010). Such associations have been reported in recent studies of several mammalian species (Hoffman et al. 2014; Huisman et al. 2016). Local effects can occur in the absence of inbreeding and thus do not necessarily imply inbreeding depression. Differentiating between general and local drivers

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of HFCs therefore depends on the presence of interindividual variation in inbreeding, which can be detected via identity disequilibrium.

Under ideal conditions, such as when investigating laboratory models and small isolated populations (Jiménez et al. 1994; Keller et al. 1994), researchers can use pedigrees to estimate the inbreeding coefficient (F), which can then be interpreted with respect to population history. However, in most wild populations pedigrees can be challenging to construct, especially if individual movements and family member associations are hard to track. Additionally, immigration and extrapair paternity make estimates of inbreeding based solely on observed pedigrees unreliable in natural populations (Chen et al. 2016). Even with a complete pedigree, the realized proportion of the genome that is IBD will differ from the expectation as a result of Mendelian sampling, recombination, and linkage (Chen et al. 2019). Another problem of estimating individual inbreeding using a pedigree is that loci can be IBD as a result of more distant ancestors than those included in the pedigree (Kardos et al. 2016).

By contrast, using genetic markers to estimate multi-locus heterozygosity is an indirect but potentially highly accurate approach to measure individual inbreeding. In the past, many published studies of HFCs were limited to a small panel of microsatellites that offer poor estimation of high-degree relationship and genome-wide heterozygosity (Balloux et al. 2004; Slate et al. 2004). The statistical power to detect inbreeding depression is therefore likely to be low, and effect size estimates of inbreeding depression may be downwardly biased (Kardos et al. 2016). Huisman et al. (2016) suggested that the prevalence of inbreeding depression may have been underestimated because studies rely on small numbers of markers or pedigrees. However, recent studies (Hoffman et al. 2014; Huisman et al. 2016; Kardos et al. 2018) employing large numbers of genetic markers maximize our ability to infer relationships and test whether inbreeding can explain HFCs (Chapman et al. 2009; Miller et al. 2014).

The recent advent of next-generation sequencing techniques has been transformative in facilitating the rapid screening of thousands of markers in natural populations of interest to behavioral ecology (see Edwards 2013; Santure et al. 2013; Chen et al. 2016). For example, double-digest restriction site-associated DNA sequencing (ddRAD-seq) is a method that can quickly yield a large number of single-nucleotide polymorphisms (SNPs) and their genotypes, even in genomically unstudied species (Andrews et al. 2016). Larger numbers of SNPs allow far more precise estimation of the genome-wide heterozygosity than is possible using a small set of microsatellites or a pedigree-based inbreeding coefficient (Keller et al. 2011; Kardos et al. 2015; Wang 2016). The increased precision of genome-wide het-

erozygosity estimation has increased the power to detect inbreeding depression and to reliably estimate its strength (Hoffman et al. 2014; Pryce et al. 2014; Béréños et al. 2016; Huisman et al. 2016). Although an intermediate number of markers may not outperform a good pedigree (Nietlisbach et al. 2017), simulations (Keller et al. 2011; Kardos et al. 2015; Wang 2016) and empirical studies of natural populations (Hoffman et al. 2014; Pryce et al. 2014; Béréños et al. 2016; Huisman et al. 2016) show that inbreeding coefficient estimates derived from a large number of genome-wide markers outperforms those based on pedigrees and therefore should allow the sensitive detection of inbreeding depression in natural populations without pedigrees (Hoffman et al. 2014; Huisman et al. 2016).

Use of genomic markers can also detect inbreeding (i.e., realized IBD) arising from very distant common ancestors (e.g., >50 generations; Keller et al. 2011), which may have fitness consequences in contemporary populations. The variation in inbreeding detected among individuals can be influenced by both mating system and demographic history (Bierne et al. 2000; Szulkin et al. 2010), and it emerges primarily in these population contexts: (1) occurrence of systematic consanguineous matings (Ohta and Cockerham 1974); (2) genetic drift or population bottleneck, in which consanguineous matings occur at random as a result of small population size (Ohta 1971; Bierne et al. 2000); and (3) admixture, in which individuals with ancestors from different populations are relatively “outbred” compared with relatively “inbred” individuals from the same population (Tsitrone et al. 2001). It has been shown that a population bottleneck or decline may lead to extensive inbreeding and inbreeding depression in endangered species with small populations (e.g., Xue et al. 2015; Abascal et al. 2016; van der Valk et al. 2019). However, there is a lack of studies connecting genetic consequences in small populations to those in large populations, probably because inbreeding and inbreeding depression is uncommon and because sampling is challenging in large populations.

The power of using a large number of genomic markers makes detection of inbreeding depression in large populations possible. Here, we applied ddRAD-seq to determine variation in inbreeding, HFCs, and mating system and both ddRAD-seq and whole-genome sequencing data to investigate the demographic history in a large population of Leach’s storm-petrels (*Oceanodroma leucorhoa*). The Leach’s storm-petrel is a long-lived, burrow-nesting pelagic seabird species distributed throughout the North Atlantic and the North Pacific. They are abundant throughout their range, with more than 20 million individuals breeding on coastal and offshore islands, where both parents incubate and provision their chick within home burrows (Mitchell et al. 2004; Bicknell et al. 2012).

Breeding colonies may contain hundreds or thousands of nesting pairs, and within these colonies Leach's storm-petrels are socially monogamous, have high mate and burrow fidelity, and lay only one egg per breeding season (Warham 1990; Bried et al. 2003). Because it is not possible to obtain complete pedigrees in a large, possibly outbred population, Leach's storm-petrels are an ideal seabird species to test the power of next-generation sequencing for resolving inbreeding depression and its cause. A previous study using minisatellites detected no extrapair paternity in this social monogamous species (Mauck et al. 1995). Here, we reexamined this possibility and evaluated the ability of ddRAD-seq-generated SNPs to accurately assign parentage, infer kinship, and determine the incidence of extrapair paternity. We then combined SNP data with extensive multiyear life-history and phenotypic trait data to investigate the potential effects of inbreeding depression. Specifically, we tested whether parental kinship and individual internal relatedness were associated with annual pair breeding success and individual breeding success, respectively, and whether internal relatedness was associated with morphometric traits of adults or chicks. We then determined whether the HFCs identified were due to general effects and asked whether the variation in inbreeding was potentially influenced by either the demographic history or the mating system. Our study reveals inbreeding depression in a large wild population and demonstrates that demographic events were the most probable underlying cause of variation in inbreeding in contemporary populations.

Material and Methods

Study Site and Sample Collection

Our study site is located at Bon Portage Island, Nova Scotia, Canada (43°26'N, 65°45'W), where 50,000 breeding pairs of Leach's storm-petrels had been previously censused (Oxley 1999). Between 2010 and 2015, we monitored approximately 550 burrows distributed across three colony sites (fig. 1) from July to September, coinciding with late incubation and chick-provisioning stages. Data from 2010 and 2015 was used only for adult morphology analysis. These data provided a demographic history of breeding partners and reproductive success for this study (Hoover et al. 2018). On first capture, each individual was banded with a unique number on its right leg for permanent identification. The age class (chick or adult), morphometric measurements (flattened wing chord, tarsus, and head-bill length), weight, and burrow number of each individual were recorded. Approximately 75 μ L of blood was taken via brachial vein and stored in a microcentrifuge tube containing Queen's lysis buffer (Seutin et al. 1991). The blood samples were stored at 4°C until DNA

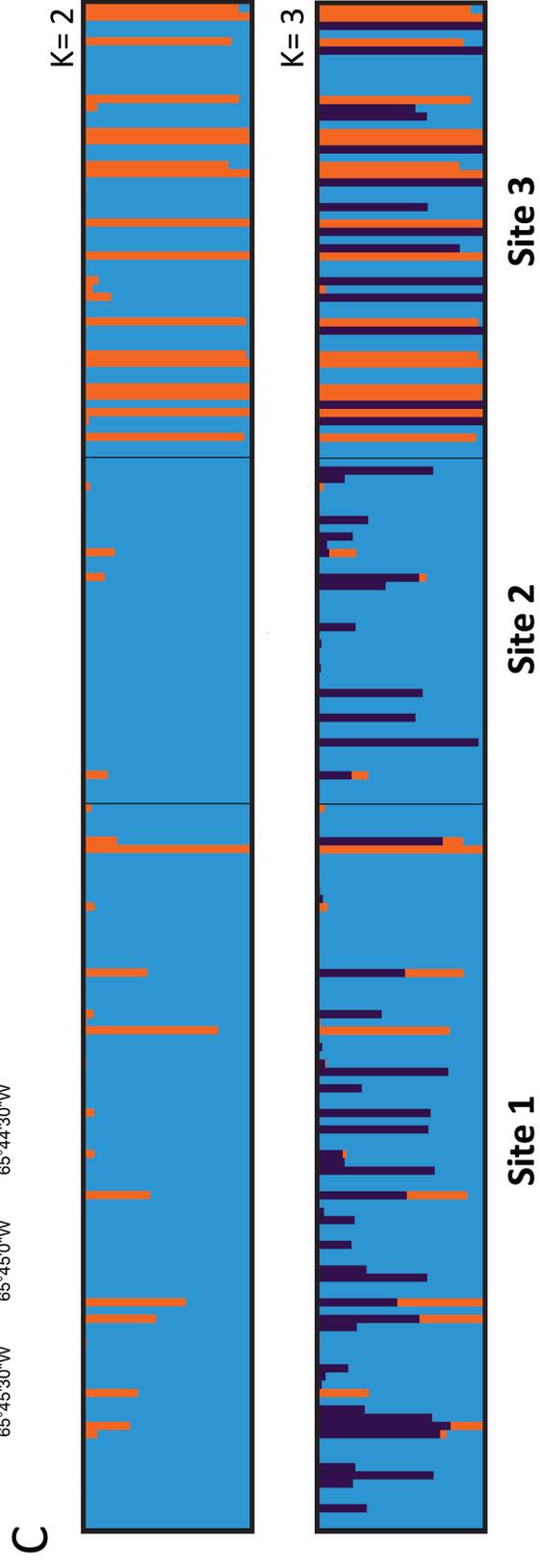
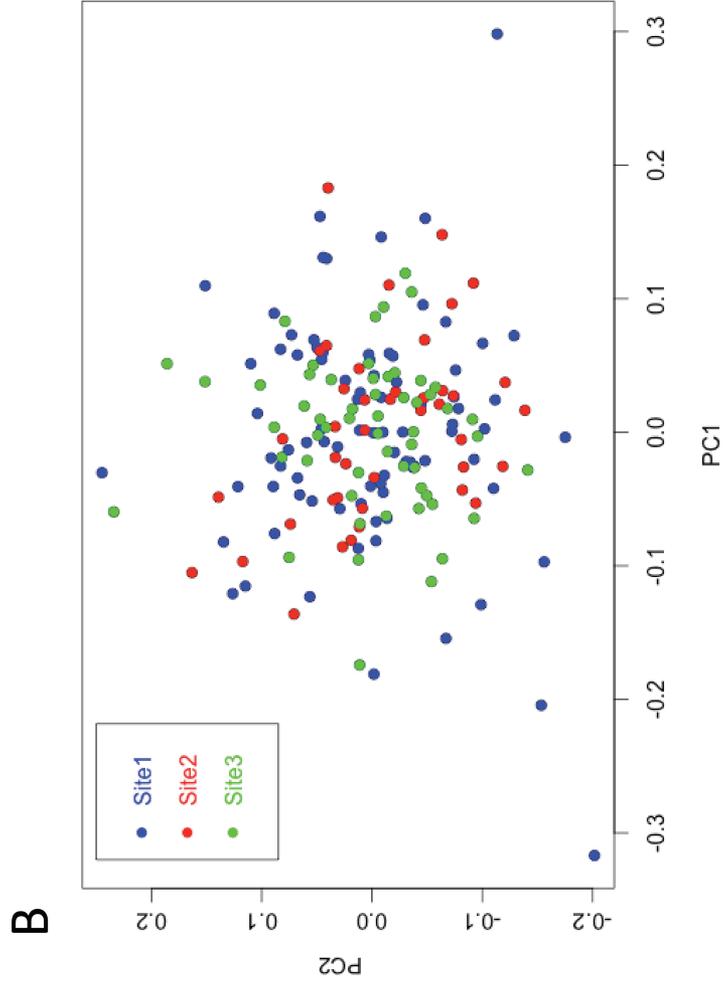
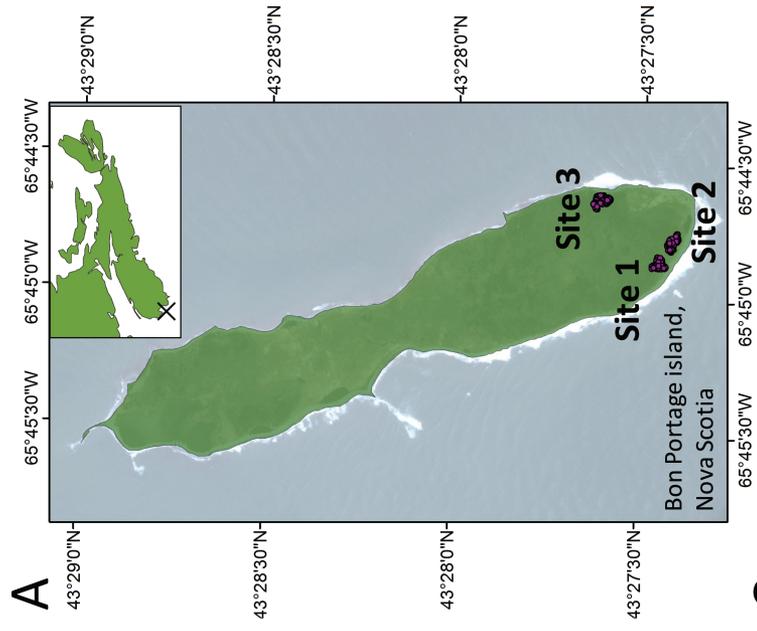
extraction. For this study, we analyzed data and samples from 560 individuals. All sampling was conducted in adherence to guidelines defined by the University of California, Davis, Institutional Animal Care and Use Committee protocol 19288.

ddRAD-seq Genotyping

DNA Extraction and ddRAD-seq. Genomic DNA was isolated using an AutoGenprep 965 extractor (AutoGen, Holliston, USA). We performed sexing polymerase chain reaction (PCR) using published primers (2550F and 2718R; Fridolfsson and Ellegren 1999). DNA concentrations were measured using the Qubit dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA) and the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen). ddRAD-seq libraries were prepared according to the protocol of Peterson et al. (2012). Restriction enzymes *SphI* and *EcoRI* were used for digestion of extracted DNA. Each bird was assigned a 5-bp inline barcode, and equimolar concentrations of 20 uniquely bar-coded individuals were pooled and double indexed via 11–14 cycles of PCR (KAPA Long Range HotStart PCR Kit; KAPA Biosystems, Wilmington, MA) with Illumina 6-bp barcodes, resulting in a unique combination of inline and Illumina barcodes for each individual. The PCR products were pooled in equimolar quantities and sequenced using high-output v4 chemistry of an Illumina HiSeq 2500 sequencer at the Bauer Core facility of Harvard University, producing 125-bp paired-end reads.

Sequence Processing. We used the program `process_radtags` in Stacks 1.30 (Catchen et al. 2013) to demultiplex the sequence reads, with the commands `-c` to clean the data by removing any reads with an uncalled base, `-q` to discard reads with low-quality scores, and `-r` to rescue barcodes and RAD tags. The paired-end reads were then merged into a single file for each individual. The reads were aligned against a draft genome of a Leach's storm-petrel (Sin et al. 2019) using Bowtie2 (Langmead and Salzberg 2012), and reads with multiple hits were excluded from the downstream analyses. The program `ref_map.pl` in Stacks was then used to create a catalog of loci and to call SNPs. We used the `populations` program in Stacks to filter and export the data for subsequent analyses. The filtering protocol for the `populations` program was one SNP per locus, 20 for minimum stack depth, and 0.05 for minor allele frequency.

Loci that deviated from Hardy-Weinberg equilibrium ($N = 139$) or that had a high null allele frequency (i.e. >0.1 ; $N = 157$) were identified using CERVUS 3.0.7 (Kalinowski et al. 2007) and were excluded by specifying a blacklist in the `populations` program (Catchen et al. 2013). To identify and exclude sex-linked markers, we used LAST (Kielbasa et al. 2011) and MUMMER (Kurtz



et al. 2004) to align the scaffolds of the Leach's storm-petrels genome to the zebra finch (*Taeniopygia guttata*) Z chromosome (National Center for Biotechnology Information). Sixty-six loci were on scaffolds mapped to the zebra finch Z chromosome, 65 of which were also identified by the Hardy-Weinberg equilibrium test. Estimates of genetic diversity (π) were also calculated using Stacks. A total of 2,427 loci from a data set of 480 individual Leach's storm-petrels were included for parentage and kinship analyses, and a total of 2,514 loci from a data set of 560 individuals (with replicates and mating pairs added to the parentage and kinship data set) were included for HFC analyses. An adult-only data set (313 individuals) comprising 3,083 polymorphic loci was used for the analysis of genetic diversity, identity disequilibrium, and linkage disequilibrium decay. We quantified the error rate of our ddRAD-seq approach by including sample replicates of 35 individuals in our library preparation and sequencing (for details, see the supplemental PDF, available online).

Data Analyses

Parentage Analyses. In this study colony, mated pairs were followed over multiple years, and most individuals remained loyal to a home burrow (Hoover et al. 2018). Thus, to predict parentage, the candidate mother and candidate father were designated on the basis of whether they were identified together in the burrow where a given chick was hatched. We confirmed parentage within each trio using CERVUS, which enables the presence of genotyping error and the proportion of unsampled individuals to be incorporated. Chicks were assigned maternity and paternity when assignment confidence was $\geq 95\%$.

We ran CERVUS simulations for 10,000 cycles, using the allele frequencies of 480 individuals. We estimated the proportion of loci that were typed incorrectly by re-genotyping 35 (7.3%) of the samples using a similar platform as the main analyses. We entered the proportion of loci mistyped as 0.1 in both the simulations and the likelihood calculations.

Kinship Inference. The software KING (Manichaikul et al. 2010) was also used to infer the kinship coefficient between individuals. KING was designed for relationship inference using high-throughput genotype data and allows for an unknown population substructure. The inferred kinship values within each trio were used to verify the parentage results from CERVUS. Kinship is around 0.25 for

first-degree relative pairs (e.g., parent-offspring, full sibling) and ~ 0.125 for second-degree degree relative pairs (e.g., grandparent-grandoffspring, half-sibling).

With the parentage results, we were able to assign relationships for parent-offspring pairs. Leach's storm-petrels are reported to begin breeding when they are approximately 5 years old (Morse and Buchheister 1977) and produce only one chick a year; thus, siblings are born in different years. Full siblings and half-siblings were subsequently identified according to the identity of their assigned parents. We determined the kinship values of these assigned relationships, parent pairs, and between-family adults (excluding parent pairs).

Inbreeding Avoidance and Analysis of Population Substructure. We also used the inferred kinship values to test whether mate choice was a consequence of selection on genomic background, via inbreeding and outbreeding avoidance, by performing randomization tests (Sin et al. 2015). This approach enabled us to compare the mean kinship value with the frequency distribution of mean values generated from 1,000 simulations of the same number of randomly selected parent pairs (see "Supplementary materials and methods" in the supplemental PDF). We then examined multidimensional scaling and STRUCTURE plots to detect any possible substructuring among our samples (see "Supplementary materials and methods" in the supplemental PDF). If there was no population structure, then a pattern of deviation from random mating in the randomization tests could be the result of inbreeding or outbreeding avoidance.

Rarefaction Analysis. We used a rarefaction analysis to calculate the number of loci required to provide consistent kinship estimates. We first randomly selected 100 loci and calculated the pairwise kinship for all parent pairs, and we then randomly selected another 100 nonoverlapping loci and calculated the pairwise kinship again. We then calculated the difference in mean kinship between these two random samples. This procedure was repeated 100 times, and the mean difference in kinship was calculated. We increased the number of sampled loci by 100 and repeated this calculation of mean difference in kinship. This procedure was repeated until 1,200 loci were selected. We then plotted the mean difference values as a function of the total number of loci drawn.

Tests for Signatures of Inbreeding. A general effect of HFCs based on multiple loci can arise only if there is

Figure 1: A, Location of three sampling sites on Bon Portage Island. The results of multidimensional scaling (B) and STRUCTURE (C) plots with $K = 2-3$ do not show any evidence of population substructuring. Colors in the STRUCTURE plot represent the estimated membership coefficients. PC = principle component.

variance in inbreeding in the population. To determine whether our SNP markers carry information about inbreeding and genome-wide levels of heterozygosity or whether inbreeding depression is a possible cause of HFCs (Kardos et al. 2014), we evaluated the identity disequilibrium (the correlation of heterozygosity across loci) by calculating the parameter g_2 (David et al. 2007) and the heterozygosity-heterozygosity correlation (HHC; Ballou et al. 2004). The parameter g_2 is an estimate of identity disequilibrium that measures the excess of standardized double heterozygosity at two loci relative to the expectation under random association (i.e., $g_2 = 0$; Szulkin et al. 2010). We computed g_2 —the probability that g_2 differs from zero (i.e., the null hypothesis of no variance in inbreeding)—with 1,000 permutations and the standard error with 1,000 bootstraps, using the *inbreedR* package (Stoffel et al. 2016). We ran an HHC test with 1,000 permutations of the marker partition using *inbreedR* (Stoffel et al. 2016). The HHC test randomly splits the loci into two subsets and calculates the average correlations between them. A significant positive correlation of heterozygosity from independent subsets of loci indicates that the heterozygosity at the markers correlates with genome-wide heterozygosity. We then explored the sensitivity of g_2 and HHC to the number of loci by randomly sampling subsets of loci from 100 to 3,000. We also investigated the predicted correlation between observed heterozygosity and inbreeding using equation (8) of Miller and Coltman (2014).

Analysis of HFCs. Throughout the sampling period, we found that certain breeding pairs returned to their burrow in multiple years but never successfully hatched a chick. We identified 21 such pairs, and we refer to them as “unsuccessful pairs” hereafter. All unsuccessful pairs were present for only 2 years in our data set, and all except two pairs were found in consecutive years. To test the hypothesis that this failure was due to mating between relatives, we compared the within-pair kinship of unsuccessful pairs ($N = 21$) with that of successful pairs ($N = 91$). Successful pairs were pairs found to always breed successfully in our data set, without records of unsuccessful breeding. Most of them were present in only 2 years or 1 year (1 year: 70 pairs; 2 years: 18 pairs; 3 years: 3 pairs). We used a generalized linear mixed model with breeding success as the response, parent pair kinship and year as the fixed effects, and pair identity as the random effect.

We also tested whether genome-wide heterozygosity as estimated by the genotyped SNPs contributed to the difference in reproductive success between successful and unsuccessful pairs. We calculated two heterozygosity estimates—standardized heterozygosity (SH; Coltman et al. 1999) and internal relatedness (IR; Amos et al.

2001)—using the *R* function *Rhh* (Alho et al. 2010) and determined whether the heterozygosity of males or females between successful and unsuccessful pairs were different. SH and IR were highly correlated ($r = -0.96$) and the results using either estimate are consistent, so we use only IR as an estimate of genome-wide heterozygosity hereafter. We used a generalized linear mixed model with breeding success as the response, together with three fixed factors and an interaction term (IR, sex, IR \times sex, and year) as well as a random factor (pair identity), to test whether IR affected reproductive success ($N = 300$).

Genome-wide IR might affect the size of adults and chicks as well as the growth rate of chicks (Chapman et al. 2009; Szulkin et al. 2010). We therefore also investigated the effect of IR on adult and chick morphology and chick growth rate. Adults were measured when they were incubating in mid-July. The measurements we took in adults were body condition (weight/tarsus length), tarsus length, and wing chord length. Chick measurements were weight, tarsus length, head-bill length, and wing chord length. Weather permitting, we measured the chicks every 3 to 4 days in the field. The growth rates are the slopes of linear functions of weight, tarsus length, or head-bill length against the number of days after hatch. Linear functions described the data well (mean R^2 of 0.85 for weight, 0.90 for tarsus length, and 0.94 for head-bill length); thus, the slopes are good estimates of the growth rate at this stage of development. Since the days on which measurements were made were not the same in all chicks, we calculated the values of the traits on day 10 based on quadratic polynomial functions (see fig. S1; figs. S1–S5 are available online; Weimerskirch et al. 2000), which show an even better fit to the data (mean R^2 of 0.93 for weight, 0.98 for tarsus length, 0.99 for head-bill length, and 0.99 for wing chord length).

We performed separate tests to determine the relationship between IR and morphometric traits for adults and chicks. We investigated the association between IR and adult traits ($N = 296$ – 298) by constructing generalized linear models with the adult parameters as response variables and IR, sex, year, and IR \times sex as fixed effects. To investigate the effect of IR on chick traits ($N = 102$ – 104), we constructed generalized linear models containing sex, year, nesting time (early, mid, or late, which are three periods equal in length from the first to the last hatch date recorded in a particular year in the data set; see table S1; tables S1–S17 are available online), genetic factors (i.e., individual IR, maternal IR, paternal IR), and biologically relevant interactions (individual IR \times sex; for contrasting HFCs among sexes, see Bichet et al. 2019) as fixed effects. We included year and nesting time to incorporate effects of temporal difference between and during breeding seasons, respectively.

We performed multimodel inference (Burnham and Anderson 2002) to establish which explanatory variables were influential, averaged over all plausible models (for details, see Sin et al. 2014, 2016). In brief, model selection was based on Akaike's information criterion corrected for sample size (AICc; Akaike 1973). Multimodel inference was performed for models with an ΔAICc of <7 (Burnham et al. 2011). We reported model-averaged parameter estimates and their 95% confidence intervals (CIs; Anderson 2008). The model-averaged estimate is a natural average of the estimate (Burnham and Anderson 2002), which averages only over models where the parameter appears. Analyses were performed using the packages *lme4* (ver. 0.999375-42; Bates et al. 2014), *MuMIn* (ver. 1.7.7; Barton 2009), and *AICcmodavg* (ver. 1.25; Mazerolle 2011) in R (ver. 3.6.3; R Development Core Team 2020).

Tests of Genome-Wide HFCs. If the HFC identified is genome-wide, then it should be strengthened by using more markers (Hoffman et al. 2010, 2014) until the markers accurately reflect genome-wide heterozygosity. In contrast, with the addition of more loci, the effect of an HFC should become weaker if it is due to local effects. We tested this prediction by generating random samples of markers ranging from 100 to 2,500 SNPs and recalculated the IR for chick, mother, and father. Then we reran the generalized linear model of chick tarsus length for each random sample to calculate the average percentage deviance explained by maternal IR (Hoffman et al. 2014). Random sampling of SNPs was carried out 10 times for each number of subsampled SNPs. Since the three traits were highly correlated on day 10 (for tarsus and head-bill length, $r = 0.87$; for tarsus and wing chord length, $r = 0.87$; for head-bill and wing chord length, $r = 0.84$), we used tarsus length only for this analysis and the following association analyses.

Allele Frequency Spectra. One of the demographic analyses requires the allele frequency spectrum as input. We used the pipeline in ANGSD (Korneliussen et al. 2014) to infer allele frequency spectrum from the ddRAD-seq reads mapped to the Leach's storm-petrel reference genome (following Dierickx et al. 2020), accounting for uncertainty in low-coverage sequencing data. Only adults were included in the analysis. Genotype likelihoods were inferred using ANGSD with likelihood method 1, and only sites with SNP quality of ≥ 20 were considered. The realSFS tool was then used to infer the folded allele frequency spectrum with a maximum of 100 iterations, with 20 bootstraps.

Inference of Demographic History. We applied two different approaches to investigate historical demographic changes in Leach's storm-petrel. First, we used the pair-

wise sequential Markovian coalescent (PSMC) model (Li and Durbin 2011) based on a draft genome of Leach's storm-petrel (Sin et al. 2019) to reconstruct the population history (see "Supplementary materials and methods" in the supplemental PDF). In the second approach, we used Stairway Plot (ver. 2; Liu and Fu 2015) to infer the optimal population size history based on the site frequency spectrum (averaged across 20 bootstrap replicates). Stairway plots are better than the PSMC model in inferring more recent population size history, while the PSMC model excels in estimating the distant past (Li and Durbin 2011; Liu and Fu 2015; Patton et al. 2019). We used the two-epoch model, with the recommended 67% of sites for training and 200 bootstraps. Following the stairway plot pipeline, we tested four different numbers of random breakpoints (161, 322, 483, and 644) to find the best grouping of θ values fitting the observed site frequency spectrum. The best number of random breakpoints was chosen on the basis of the training data.

To convert inferred population sizes and times to numbers of individuals and years, respectively, we used the mutation rate estimate of 2.89×10^{-9} per site per generation from the northern fulmar (*Fulmarus glacialis*; Nadachowska-Brzyska et al. 2015). We estimated the generation time of Leach's storm-petrel as the age of sexual maturity multiplied by a factor of two (Nadachowska-Brzyska et al. 2015), which gave a generation time of 10 years.

Results

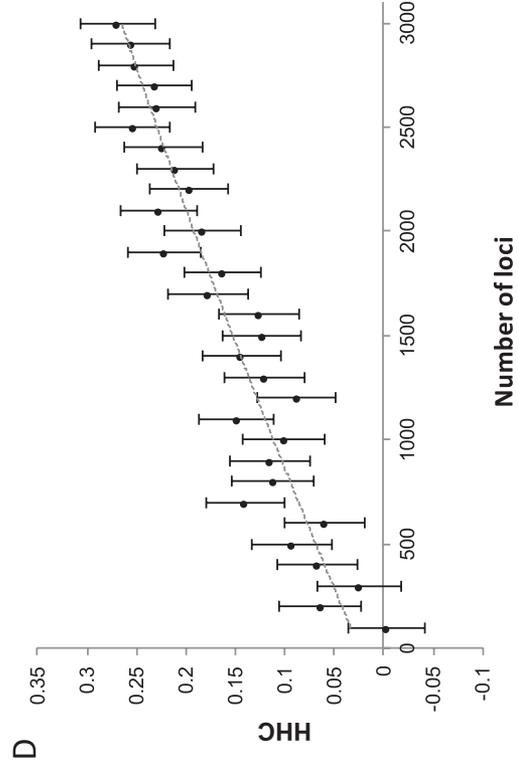
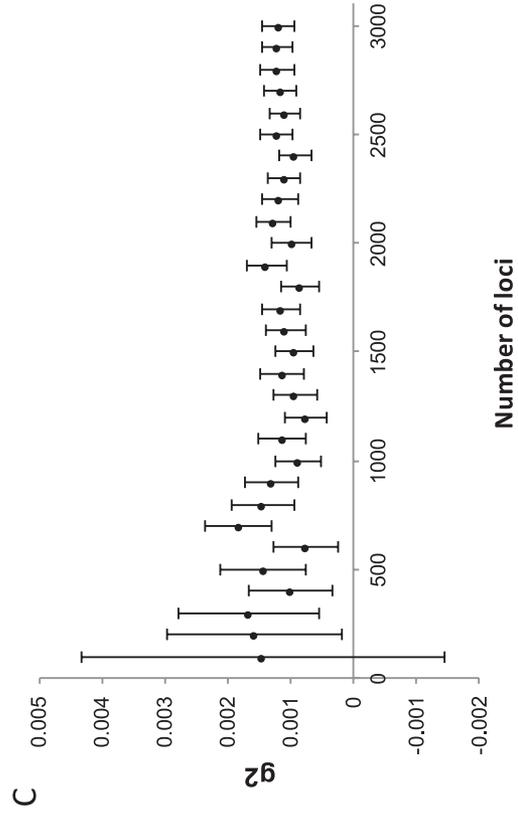
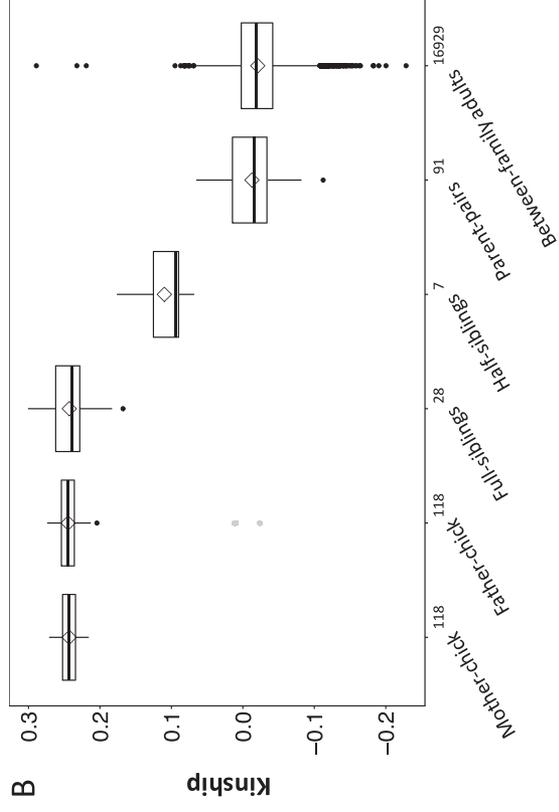
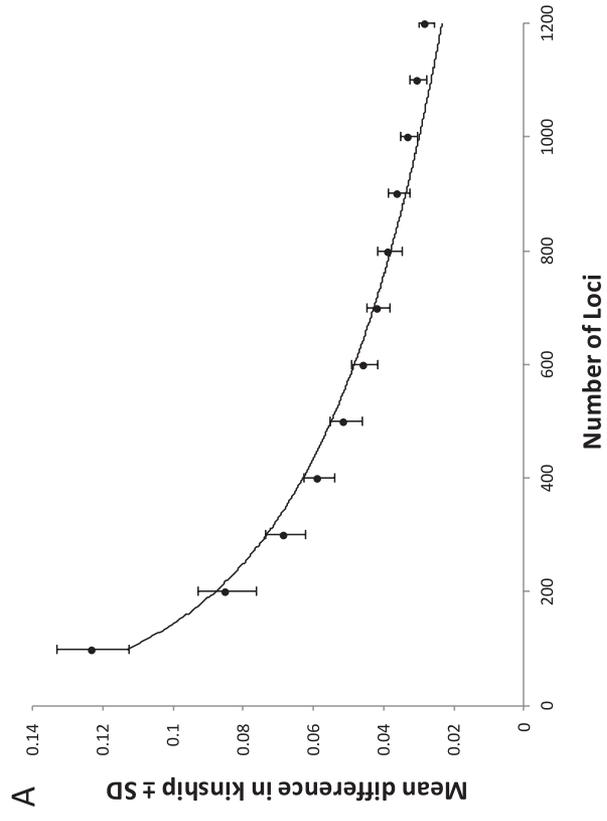
Parentage

There were 2,427 polymorphic RAD loci retained for the parentage and kinship analyses across a total of 480 birds (details are provided in "Characterization of RAD data" in the supplemental PDF). The mean proportion of loci typed was 0.89, and the mean individual heterozygosity was 0.30. The nonexclusion probabilities (table S2) indicated a high power for confirming parentage.

Both parents were assigned to chicks for 123 (97.6%) trios. Only maternity was assigned to chicks for the remaining three trios, which suggests that the candidate father from the same burrow was not the genetic father. Extrapair paternity was found only in two (2012 and 2013) of the four study years (2011 to 2014). The number of trios, number of extrapair paternity events, and the rate of extrapair paternity with respect to the 4 years we examined are given in table S3. The overall rate of extrapair paternity was 2.4% (3/126 trios).

Kinship

Rarefaction analysis indicated that the reliability of kinship inference increased rapidly as more loci were added



Dyads of assigned relationships

to the data set (fig. 2A). The mean pairwise kinship difference among pairs of individuals in a given relationship class dropped to less than 0.03 with 1,200 loci. The data set of 2,427 loci therefore provided a highly reliable estimation of kinship (i.e., the kinship difference is 0.0 when using 2,427 loci, according to the equation in the legend to fig. 2A).

The results from kinship analysis agreed with the parentage result from CERVUS. The mean kinship was ~ 0.25 (SE = 0.0008) for all parent-offspring dyads ($N = 236$; fig. 2B), except for the three instances in which the resident male did not father the resident chick (mean kinship between male and chick = -0.001 ; SE = 0.01). The average kinship between mated pairs was -0.01 (SE = 0.003; fig. 2B), indicating that on average pairs were composed of unrelated individuals. We did not identify any pairing of close relatives (i.e., first-degree [~ 0.125] or second-degree [~ 0.0625] relatives). We identified three first-degree relatives between adults from different pairs in our data set. The remaining kinship values were clustered around zero (fig. 2B), indicating that most parent pairs were unrelated to each other. Using the kinship data, we identified no overall deviation from random mating across the 4 years examined based on the genomic background, but parent pairs were found to have higher kinship values than expected under random mating in 1 year (supplemental PDF, “Supplementary results”).

Population Substructure

When comparing individuals across all three sampling sites, the Evanno method (Evanno et al. 2005) identified two clusters ($\Delta K = 5,319$) as more optimal than three clusters ($\Delta K = 2,742$). We note, however, that the Evanno method cannot identify a single cluster as a superior model to multiple clusters, leaving open the possibility that one cluster is indeed optimal. The STRUCTURE results showed no evidence of clustering with respect to the three sampling sites in the Bon Portage Island breeding population (fig. 1C). Similarly, the multidimensional scaling analysis using KING does not show any evidence of population substructuring, as individuals from different sites were mixed together (fig. 1B). The mean kinship value was significantly different from expectation from

random mating in 2013 (supplemental PDF, “Supplementary results”; figs. S2, S3). We therefore examined whether this result was driven by clustering of closely related individuals using a separate STRUCTURE analysis for this year but did not find any evidence of substructuring (data not shown).

Inbreeding Depression

A signature of interindividual variation in inbreeding (identity disequilibrium) was present in the Leach’s storm-petrel RAD data. The mean HHC was 0.266 (SD = 0.038, 95% CI = 0.191 to 0.338), and the parameter g_2 was significantly different from zero (0.0012, SE = 0.00025, $P < .002$). This was the case even with as few as $\sim 1,500$ SNPs (fig. 2C), which was also sufficient to detect a strong positive HHC (fig. 2D). These results suggest that heterozygosity was significantly correlated across loci and that our SNP markers can reliably estimate genome-wide heterozygosity and test the general effect of HFC (Szulkin et al. 2010). The correlation between observed heterozygosity and inbreeding was 0.50. The estimate of genetic diversity (π) as calculated by Stacks was 0.265 when examining polymorphic loci only and 0.00062 when examining both variant and invariant loci.

Adult Reproductive Success and Morphology. As expected, because parent dyads were generally unrelated, parental kinship failed to predict breeding success for mated pairs. Individual IR also failed to predict individual breeding success (tables S5, S6). There was no association between adult individual IR and any adult morphological trait we measured, which included weight, tarsus length, and wing chord (tables S7–S9).

Chick Growth Rate and Morphology. In the chicks with known parentage, we found no association between individual chick IR and growth rates (tables S10–S12). However, there was a strong trend for chicks with more inbred (i.e., more homozygous) mothers to have lower weights (maternal IR: $\beta \pm SE = -43.53 \pm 17.39$, 95% CI = -77.61 to -9.46 ; table S13). Increased maternal IR was also strongly associated with smaller values for the morphological traits we measured in chicks (fig. 3). We found

Figure 2: A, Relationship between the number of loci used and the mean difference in kinship between nonoverlapping sampling. The curve is described by the function $y = -0.036 \ln(x) + 0.2793$, $r^2 = 0.973$. Error bars indicate ± 1 SD. B, Kinship of dyads of relationships observed in the field in the same nest and assigned using CERVUS. Parentages were assigned with $\geq 95\%$ confidence. Parent pair indicates assigned parents of the same chick. Full siblings and half-siblings were chicks that hatched in different years yet had the same assigned parent and assigned mother or assigned father, respectively. Between-family adults were all dyads between adults excluding parent pairs. The numbers below the X-axis are the number of dyads, and the box represents the interquartile range. The line inside the box indicates the median, and the diamond indicates the mean. The upper whiskers extend to the highest value within 1.5 times the interquartile range, and the lower whiskers extend to the lowest value within 1.5 times the interquartile range. Black circles are outliers. The three gray circles are dyads from nests with extrapair paternity. C, Relationship between the number of randomly sampled loci and g_2 ($\pm SEM$). D, Relationship between the number of randomly sampled loci and the mean ($\pm SD$) heterozygosity-heterozygosity correlation (HHC) coefficients.

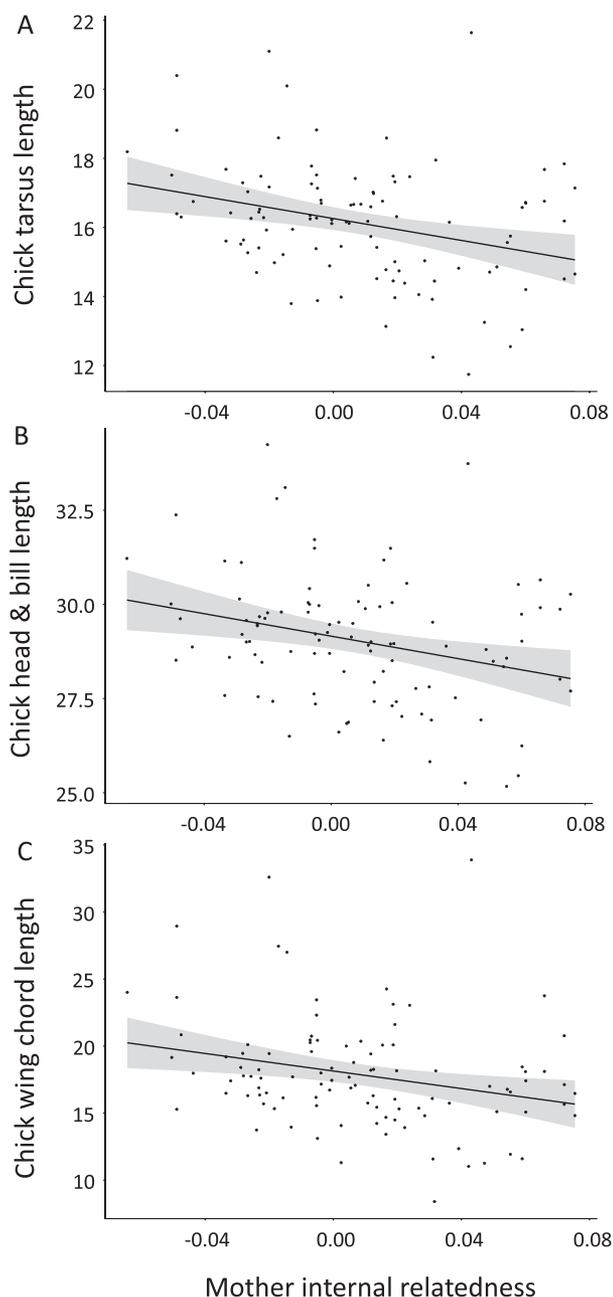


Figure 3: Relationship between maternal internal relatedness (IR) and chick morphological traits at 10 days posthatching. Chick tarsus length (A; mm), chick head and bill length (B; mm), and chick wing chord length (C; cm) decrease with the maternal IR. Lines show fitted models. Shaded areas show 95% confidence intervals.

that chicks with more inbred mothers had shorter tarsus length (maternal IR: $\beta \pm \text{SE} = -16.07 \pm 4.61$, 95% CI = -25.103 to -7.03), head-bill length (maternal IR: $\beta \pm \text{SE} = -14.52 \pm 4.98$, 95% CI = -24.287 to -4.749), and wing chord length (maternal IR: $\beta \pm \text{SE} = 0.10 \pm 0.04$, 95% CI =

0.031 to 0.171 [gamma-transformed data]; estimates for all effects are given in tables S14–S16). Maternal IR was the only genetic factor to significantly affect these morphological traits in chicks.

According to the models (tables S14–S16), chicks with more inbred mothers, with IR = 0.075 (i.e., the highest value in our data set), had tarsus lengths that were shorter on average by 2.23 mm compared with chicks with more outbred mothers (taken as IR = -0.064 , i.e., the lowest value in our data set). This constitutes a 13.9% decrease relative to the mean tarsus length of 16.02 mm at day 10 posthatching. Chicks from inbred mothers (IR = 0.075) also had head-bill lengths 2.02 mm shorter than chicks with more outbred mothers (e.g., IR = -0.064), constituting a 7.0% decrease relative to the mean head-bill length of 28.81 mm at day 10. Chicks from inbred mothers had wing chords on average 4.28 cm shorter than chicks from outbred mothers, resulting in a 23.8% decrease relative to the mean wing chord length of 18.0 cm at day 10.

Because the three traits were highly correlated at day 10, we also performed a principle component analysis to combine the measures into a single principle component (PC). The first PC (PC1) explains 92% of the variance, providing a good estimate of chick body size. Consistent with the results given above, the general body size (i.e., PC1) was significantly associated with maternal IR (table S17).

General Effect of HFC. As expected by general effects of HFCs, the average percentage deviance explained by maternal IR in the GLM of chick tarsus length increased with SNP number to 9% with 2,500 SNPs (fig. 4). The percentage deviance increased rapidly when beginning at a low number of SNPs and started to level off at $>2,000$ SNPs.

Demographic History

The PSMC analysis (fig. 5A) revealed a fluctuation in the effective population size between 120,000 and 180,000 from 100,000 to 10,000,000 years ago, with a population contraction at around 100,000 years ago, from $\sim 160,000$ individuals to below 50,000, a reduction of $\sim 70\%$. The inferred history from the stairway plot (fig. 5B) shows a substantial population bottleneck at around 200,000 years ago, where the population size dropped from $\sim 3,000,000$ to $\sim 4,000$ in $\sim 1,000,000$ years and returned to $\sim 8,000,000$ within 50,000 years, after which the population size declined gradually from $\sim 8,000,000$ to $\sim 30,000$ between 40,000 and 100 years ago, more than a 200-fold decrease.

Discussion

Using ddRad-seq, we found signatures of variation in inbreeding and inbreeding depression in Leach's storm-petrel,

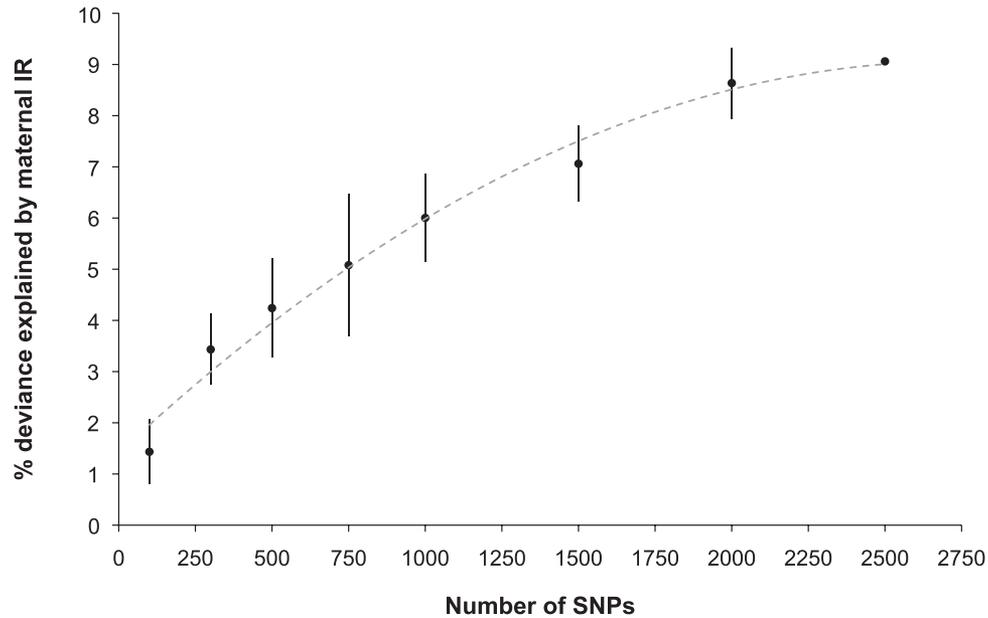


Figure 4: Relationship between the number of randomly sampled single-nucleotide polymorphisms (SNPs) and the mean (\pm SEM) percentage deviance explained by maternal internal relatedness (IR) in the generalized linear model of chick tarsus length. Random samplings of SNPs were carried out 10 times for each number of subsampled SNPs.

a common, socially monogamous seabird. The variation in inbreeding found in Leach's storm-petrel allows us to detect inbreeding depression in chick size due to high maternal IR, as a result of the general effect of HFCs. Variation in inbreeding is usually found in small populations. However, we were able to detect variation in inbreeding and inbreeding depression in a large, outbred population of seabirds, despite an absence of population structure and a socially monogamous mating system. This finding raises the possibility that demographic events, rather than mating system, underlie the signatures of inbreeding in this population.

Variation in Inbreeding

The SNP marker set used here provides enough power to reflect genome-wide heterozygosity or inbreeding, shown by significant identity disequilibrium (Szulkin et al. 2010). By examining 50 studies representing 45 species and 105 populations, Miller and Coltman (2014) detected a low average g_2 value of 0.0069 in these populations, with only 20% of the values significant. Among significant g_2 values, the mean was 0.025 (range = 0.0009–0.16). The g_2 value of Leach's storm-petrel is therefore at the low end compared with other species or populations also showing a signature of variation in inbreeding. Surprisingly, the identity disequilibrium of this population was at a level comparable

to a wild population of red deer (*Cervus elaphus*) on the Isle of Rum, Scotland (both $g_2 = 0.0012$; Huisman et al. 2016). Given that the red deer population size on Rum is smaller than 1,300 (Daniels 2006) and the red deer has a polygynous mating system, they are expected to have much greater variance in inbreeding than Leach's storm-petrel, which has a very large population size and a nearly genetically monogamous mating system. In addition to the variation in inbreeding we identified, the expected correlation between marker heterozygosity and inbreeding in this study (0.5) is higher than that of most other species reported (mean = 0.13, range = 0–0.82; Miller and Coltman 2014). This correlation also suggests that any HFC identified could be caused by inbreeding depression (Kardos et al. 2014).

Variation in inbreeding can be influenced by the mating system and by demographic history (Bierne et al. 2000; Szulkin et al. 2010). Identity disequilibrium could arise from departures from random mating, such as inbreeding (Ohta and Cockerham 1974). Although parent pairs were found to have higher kinship values than expected under random mating in one of four examined years, there was no inbreeding between close relatives found in this study, and most sexually mature adults were nonrelatives. We also showed that the mating system was nearly genetically monogamous (with a very low rate of extrapair paternity), which was not likely to generate a

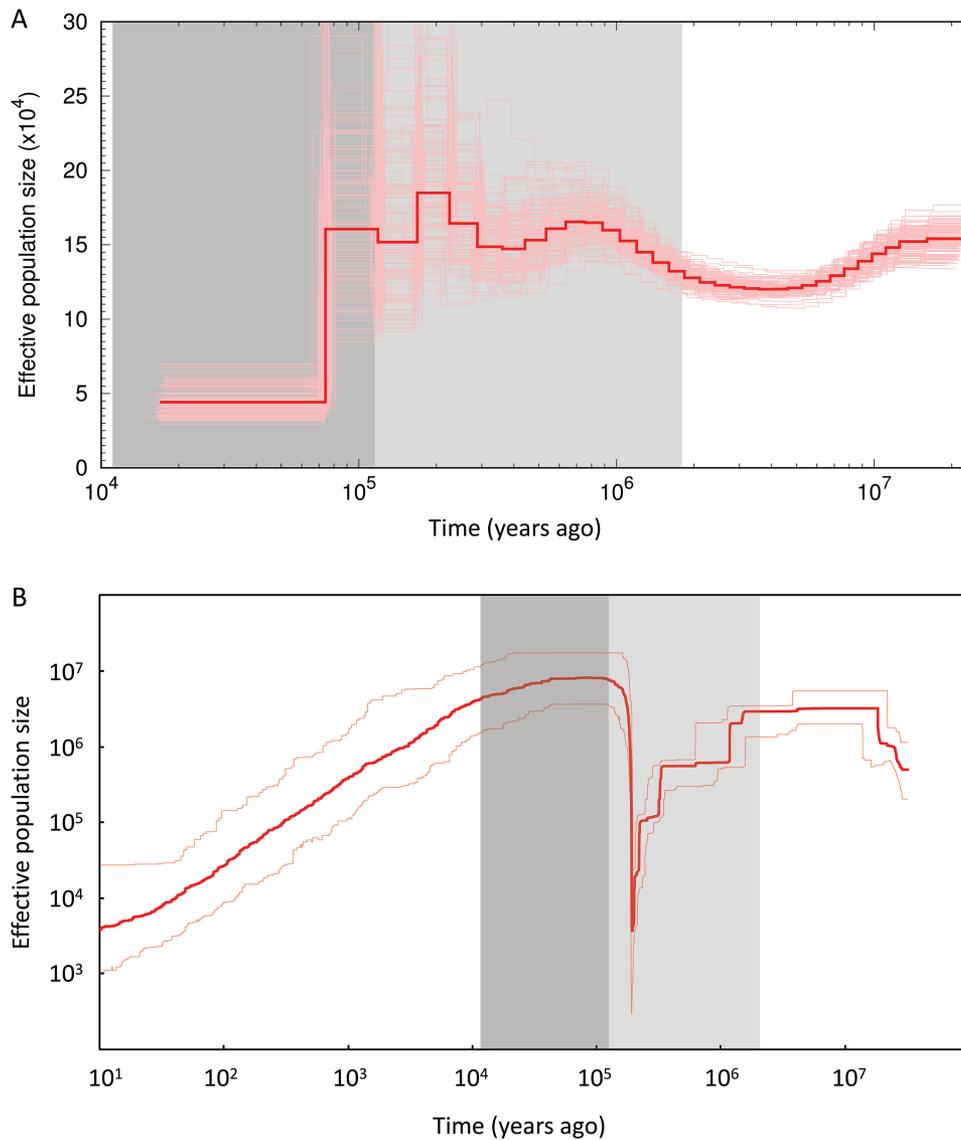


Figure 5: Demographic history of Leach's storm-petrel inferred using the pairwise sequential Markovian coalescent (PSMC) model (A) and the stairway plot method (B). For the PSMC model (A), the bold red line is the median effective population size estimate, whereas the thin lines are 100 individual bootstrap replicates. In B, the solid red line shows the median estimate, whereas the thin lines indicate the upper and lower bounds of the 95% confidence interval from 200 bootstrap replicates. In both plots, the light gray areas indicate the Pleistocene epoch, and the dark gray areas indicate the Last Glacial Period.

covariance in heterozygosity among markers. By contrast, mating systems with a few individuals monopolizing the available mates should have a relatively smaller effective population size and higher chance of genetic drift. The mating system in this population was therefore not likely to cause the variation in inbreeding identified.

Alternatively, past demographic events might explain the signature of individual inbreeding in Leach's storm-petrel (Bierne et al. 2000; Szulkin et al. 2010). Both the PSMC model and the stairway plot reveal a gradual pop-

ulation contraction starting less than 100,000 years ago from an effective population size of 160,000 or more to below 50,000. Furthermore, the stairway plot, known to be more accurate in estimating recent demographic history compared with the PSMC model (Li and Durbin 2011; Liu and Fu 2015; Patton et al. 2019), suggested a drastic population bottleneck around 200,000 years ago. A finite population having undergone a population bottleneck or decline would in theory increase variation in inbreeding due to the occurrence of consanguineous

matings and variable degree of relatedness among individuals, a trend that may persist in the population once it has recovered to prebottleneck size (Bierne et al. 2000; Szulkin et al. 2010). The inferred historical bottleneck, together with a more recently declining effective population size, might lead to an increase in identity disequilibrium in the contemporary Leach's storm-petrel population. Moreover, the Pacific and Atlantic populations of Leach's storm-petrel are believed to be genetically distinct (Bicknell et al. 2012; Hoover 2018), with evidence of small amounts of admixture, which also could generate variation in individual relatedness and signatures of inbreeding. Bicknell et al. (2012) estimated a rate of gene flow of 1.96 female migrants per generation from the Pacific to the Atlantic population, on the basis of 18 microsatellite loci. A separate analysis involving thousands of SNPs revealed gene flow in both directions at 20 individuals per year recruited from one side to the other (Hoover 2018). Both estimates represent low levels of admixture that could generate variance in inbreeding. Indeed, the negative IR values suggest relatively "outbred" individuals (Amos et al. 2001), and a negative kinship estimate implies that individuals are less related than the average (Wang 2014). It is plausible that these outbred individuals were generated by demographic processes such as admixture of Pacific and Atlantic populations and population bottlenecks rather than mate choice, because we did not identify any inbreeding avoidance. Although we did not detect any genetic substructure in this population—an unsurprising result given that migrants are uncommon (Bicknell et al. 2014)—"hybrids" from matings between local individuals and those from the Pacific might have lower IR than those from local pairs. The two types of demographic events, population bottlenecks and admixture, are not mutually exclusive in generating the variation in inbreeding. Further studies, including more detailed analyses of admixture and population size change in the past, will help us to elucidate how the pattern arose.

Maternal Inbreeding Depression

Individuals with different levels of inbreeding allowed us to detect and examine the potential consequences of inbreeding depression in offspring. We found that inbred mothers (i.e., mothers with high genome-wide IR) had smaller chicks. This result is consistent with the idea that maternal inbreeding can negatively influence allocation of resources to offspring through egg quality or parental care. For example, it is well established in birds that maternal investment in eggs can influence all stages of the offspring life cycle (Krist 2011). Less inbred (i.e., more heterozygous) mothers may be able to allocate more resources to produce larger clutch sizes (Ortego et al. 2007;

Olano-Marin et al. 2011; Wetzel et al. 2012), larger eggs (Wetzel et al. 2012), or higher egg quality (García-Navas et al. 2009). Having an inbred mother has also been shown to have negative effects on hatching rates (Sittmann et al. 1966; van Noordwijk and Scharloo 1981), morphological traits (García-Navas et al. 2014), fledging rates (Amos et al. 2001), and offspring survival (Richardson et al. 2004; Brouwer et al. 2007). The difference in the timing of expression of inbreeding depression between different species depends on life history, mating system, or constraints of the environment (Marr et al. 2006; Brouwer et al. 2007). For example, passerines tend to have many eggs per clutch, and inbred females may experience reduced reproductive success and hatching rates (van Noordwijk and Scharloo 1981; Reid et al. 2014) if they are not able to allocate enough resources to all eggs. In contrast, procellariiforms, such as storm-petrels, invest in only one offspring per breeding season; therefore, the effect of maternal inbreeding may be less dramatic on hatching rates compared with chick body size (this study) or fledging rates (Amos et al. 2001). Since storm-petrel chicks are provisioned by both parents yet only maternal but not paternal IR was associated with chick size, maternal investment in eggs could influence chick size more than parental care during the chick stage. Alternatively but not mutually exclusively, the association between IR and chick size could be due to hybrid vigor, as a result of admixture between individuals from the genetically distinct Pacific and Atlantic populations (Bicknell et al. 2012; Hoover 2018).

Life-history traits such as survival and breeding success, as well as traits such as juvenile growth rate or juvenile size (Szulkin et al. 2010), are expected to correlate with heterozygosity because they are affected by many loci and exhibit directional dominance (Szulkin et al. 2010), meaning they have many targets for deleterious recessive mutations. By contrast, adult morphological traits such as adult size, which may be under stabilizing selection and display less directional dominance, are likely to be influenced by fewer loci compared with life-history traits (Price and Schluter 1991; Chapman et al. 2009). Adult morphological traits are thus expected to be less correlated with heterozygosity than life-history traits (Coltman and Slate 2003; Chapman et al. 2009) and possibly growth traits. These ideas are supported by our results reporting no effect of IR on phenotypic traits in adults yet a significant IR effect on growth metrics (juvenile size) of chicks. Meta-analyses (Coltman and Slate 2003; Chapman et al. 2009) show that stronger effect sizes were found for life-history traits than for morphological and physiological traits such as parasite load. Indeed, inbreeding depression is generally found in traits more closely associated with fitness (DeRose and Roff 1999; Kristensen et al. 2010; Forstmeier et al. 2012; Huisman et al. 2016). For example, in song sparrows,

inbreeding influenced offspring survival after independence (Keller 1998). In our study of Leach's storm-petrels, maternal inbreeding was associated with reduced growth traits of chicks, which may incur costs during later life-history stages, such as fledging rate, survival, and lifetime breeding success.

A difference in heterozygosity between adults and chicks is expected if there is differential survival for birds with different levels of heterozygosity. We could not detect any difference in IR between adults and chicks (Welch's t -test: $t = 1.54$, $df = 402$, $P = .12$), suggesting that there was no obvious effect of individual inbreeding depression on survival. However, we were not able to determine the age of adults, so subtle differences in individual heterozygosity among birds of different ages might still exist. Although there was no effect of genome-wide heterozygosity on annual breeding success, discrepancies between inbreeding effects on annual and lifetime measures of fitness can be found in some species (Huisman et al. 2016). For example, some species of albatross may breed every 1 or 2 years, depending on the body condition of the female. It is therefore important to consider the entire life cycle of an individual to determine breeding success. Leach's storm-petrels are long-lived birds (maximum observed life span = 36 years; Haussmann et al. 2007) with a wide geographical range, making it difficult to determine survival rates and lifetime breeding success. Collecting data across longer time spans would shed light on whether maternal inbreeding can also affect adult size and survival. The maternal effect on chick size could also be nongenetic and be influenced by interactions with environmental conditions during the development of the mother.

General Effect of HFCs

Our results show that the HFCs identified were more likely to result from general effects of inbreeding rather than genomically local effects. Increasing the number of SNPs strengthened the HFCs (the deviance in chick tarsus length explained by maternal IR increased from around 1.5% with 100 SNPs to 9% with 2,500 SNPs). This result is consistent with predictions of genome-wide rather than local effects of inbreeding depression (Hoffman et al. 2014). Local effects would be predicted if the relationship between heterozygosity and fitness became weaker with increasing numbers of SNPs used in the analysis (Hoffman et al. 2010), provided that the locus causing the effect or markers linked to it are included in the analysis.

Meta-analyses have shown that effect sizes of HFCs are usually very weak, and the overall percentage of variance in fitness that heterozygosity explains is low (with

an average of 1% or less) when using microsatellites (Coltman and Slate 2003; Chapman et al. 2009). Detecting small effect sizes of HFCs requires high statistical power. The power to detect effects of heterozygosity on fitness is higher when using thousands of RAD markers compared with a small panel of microsatellites (Hoffman et al. 2014), because the estimation of interindividual variation in inbreeding coefficients is more accurate. In this study, when we analyzed a large number of SNPs, the deviance in traits explained (at ~9%) by maternal IR was higher than when using smaller numbers of markers. It is possible that previous studies that found no or only small effects of HFCs using microsatellites might have identified larger effects by using a large number of SNPs if there were genetic structures (i.e., variance in inbreeding coefficients) required to generate HFCs (Szulkin et al. 2010). Even when HFCs are detected, in most studies it is difficult to distinguish whether general or local effects are operating (Brouwer et al. 2007; Robinson et al. 2013; Santure et al. 2013) if only a small number of markers are used. These two mechanisms describe how fitness loci displaying different magnitudes of directional dominance or overdominance are distributed in the genome (Szulkin et al. 2010) and how neutral markers are related to them. General and local effects are not mutually exclusive and can exist in the same population. With the advent of high-throughput sequencing techniques, a large number of markers can be used to determine the number of loci causing inbreeding depression and the individual effect sizes of those loci. This question of the number of loci and their effect sizes for inbreeding depression is more relevant nowadays, given the power to genotype a large number of markers simultaneously.

Leach's Storm-Petrels Are Not Always Genetically Monogamous

Leach's storm-petrels are socially monogamous and, in a study of 42 trios using DNA fingerprinting, were suggested to be genetically monogamous (Mauck et al. 1995). Yet using ddRAD-seq to examine three times the number of trios, we identified three instances of extrapair paternity within our study sites. Although many procellariiforms are known for their mate fidelity, a low incidence of extrapair paternity is not uncommon for this order (see table 1 in Burg and Croxall 2006). For example, extrapair paternity has been detected in six out of 10 procellariiform species examined, using multilocus DNA fingerprinting or microsatellites. The extrapair paternity rate ranged from 4% in the black-browed albatross (*Thalassarche melanophris*) to 25% in the waved albatross (*Phoebastria irrorata*). We show that the rate of extrapair paternity in Leach's storm-petrel is 2.4%, the lowest among procellariiform

species exhibiting extrapair paternity (Burg and Croxall 2006). Our results suggest that extrapair paternity should be reexamined with higher-resolution methods in those procellariiform species where no extrapair paternity was found (e.g., *Fulmarus glacialis*, *Calonectris diomedea*, and *Oceanites oceanicus*; Burg and Croxall 2006); these species may actually have very low rates of extrapair paternity, as we found here.

We further examined the three instances of extrapair paternity to better understand their significance to the Leach's storm-petrel breeding system. In this species, males make a significant investment in constructing a burrow that houses the nest, but it is not known whether mating occurs in the nest or at sea. In two of the three instances, we found that extrapair paternity occurred in a year when the male nest occupant was transitioning to a new mate. We could not determine this information for the third male because we do not have records of this individual for years before the identified extrapair paternity. As far as we could establish, males raised chicks that were not their own when they were transitioning to a new partner. In one instance, the male stayed with the new partner, and together they reared chicks sired by that male in subsequent years. In the other two instances, the male switched partners again in a subsequent year. Taken in context, these results suggest that this transitional period allows both sexes to select and assess new partners on the basis of behavioral as well as genetic compatibilities of the major histocompatibility complex genes (Hoover et al. 2018). Our analysis has not only revealed a significant level of extrapair paternity in a species thought to be genetically monogamous but, coupled with our extensive monitoring program, has also enabled us to place this result in the behavioral ecological context of the species (see also Hoover et al. 2018).

Reduced Representation Sequencing Is a Valuable Tool to Study Parentage and Inbreeding Depression

We have shown that ddRAD-seq is a powerful tool to accurately assign parentage, infer relationships, estimate inbreeding, and determine demographic history in a large wild seabird population. ddRAD-seq is known to provide estimates of heterozygosity that are downwardly biased because of the possibility that one of the restriction sites required for detecting a locus could have mutated in highly heterozygous individuals (Arnold et al. 2013; Cariou et al. 2016). This bias could in principle influence our estimates of HFCs. However, this bias is not particularly pronounced in species where the level of polymorphism is low (Cariou et al. 2016); in the case of Leach's storm-petrel, genetic diversity is relatively low (less than 2%), suggesting that this bias is not strong. Additionally, we were able to detect and

exclude putative null alleles, thereby removing this potential source of bias. Our analyses of heterozygosity focused on relative heterozygosity among individuals, and our estimate of identity disequilibrium, a measure of the excess of standardized double heterozygosity at two loci is, if anything, likely to be underestimated by this bias. We therefore believe that the signature of variation in inbreeding we have detected is likely real.

The kinship coefficients we calculated generally reflect the relationships inferred from fieldwork, and the resolution is high for both first- and second-degree kinship. The reliability of kinship estimates increases rapidly with the number of SNPs; approximately 2,400 SNPs could reliably estimate kinship in this large population. Whereas it is time-consuming to develop and screen a large number of microsatellites, next-generation sequencing technologies (e.g., ddRAD-seq) provide a fast and high-throughput solution to develop a large number of loci in nonmodel species without genomic resources such as a reference genome (Holleley et al. 2015; Andrews et al. 2016). Targeting thousands to tens of thousands of loci can be achieved by simply changing the choice of restriction enzymes used in ddRAD-seq (Peterson et al. 2012). Researchers can thus choose whether they want to invest in either sequencing more loci or collecting more samples, depending on the project objectives.

Although on average a microsatellite locus is more variable than an individual SNP and screening of low numbers of SNPs may yield a low correlation of heterozygosity and inbreeding coefficients (DeWoody and DeWoody 2004; Santure et al. 2010), screening several thousand SNPs should capture most of the inbreeding signal in many systems (Hoffman et al. 2014; Miller et al. 2014). The number of loci required to capture this signal will depend on the extent of variation in inbreeding. That we were able to estimate kinship and inbreeding depression in a highly mobile population of one of the most numerous seabird species in the world suggests that ddRAD-seq will be promising for studies in other outbred systems. Our findings also demonstrate a promising approach for studying inbreeding and its effects in nonmodel species that are difficult to study in the wild.

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Statement of Authorship

S.Y.W.S., G.N., and S.V.E. designed the research; S.Y.W.S. and B.H. performed the research; S.Y.W.S. analyzed data; S.Y.W.S. wrote the manuscript; and all authors contributed to revised versions.

Data and Code Availability

The ddRad-seq genotyping data, fitness measures information, and bioinformatics scripts are available via the Dryad Digital Repository (<https://doi.org/10.5061/dryad.sn02v6x3c>; Sin et al. 2021).

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“In their most common type the mounds may be described as rounded eminences, or knolls, rising from one to four feet above the surrounding surface or the depressions between them, and ranging from ten to fifty feet in diameter. They are generally nearly circular and distinct, but are, in some instances, confluent or elongated.” From “The Hillocks or Mound-Formations of San Diego, California” by G. W. Barnes (*The American Naturalist*, 1879, 13: 565–571).