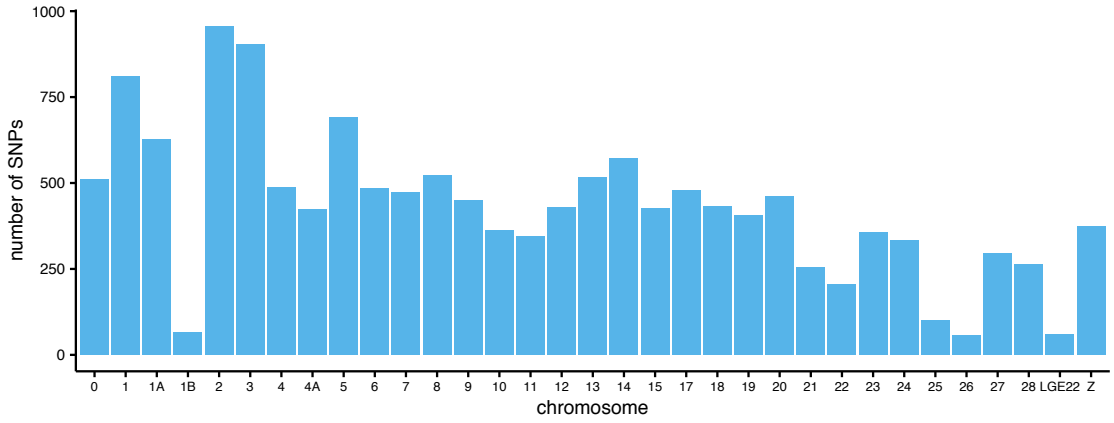


**Figure S1.** Related to Experimental Procedure. Putative chromosome locations of custom Beadchip SNPs. Putative genomic location was determined by aligning reads to FSJ genome scaffolds, which were aligned to the Zebra Finch genome. Here we show the number of SNPs aligned to each Zebra Finch chromosome. Chromosome 0 contains scaffolds that were not successfully assigned to a chromosome.

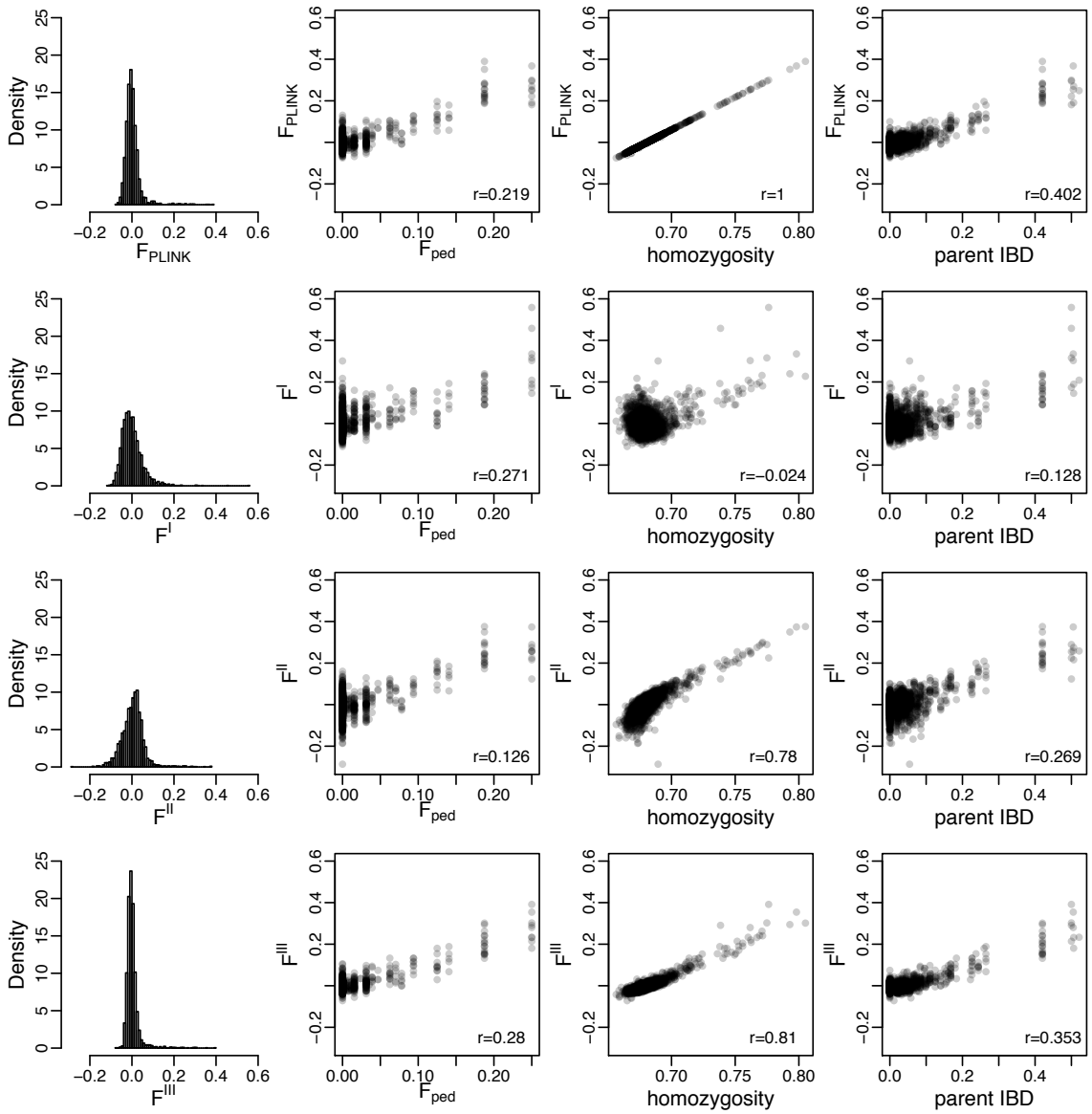


**Figure S2.** Related to Experimental Procedure and Figure 2. Comparison of different genomic estimators of inbreeding coefficient. Distributions of different inbreeding coefficient estimators ($F_{PLINK}$, $F^{I}$, $F^{II}$, and $F^{III}$) and their relationship with pedigree-based inbreeding coefficient ($F_{ped}$), average SNP homozygosity, and pairwise IBD between the parents for 2,087 nestlings with two genotyped parents. Correlation coefficients shown are Spearman's rho.

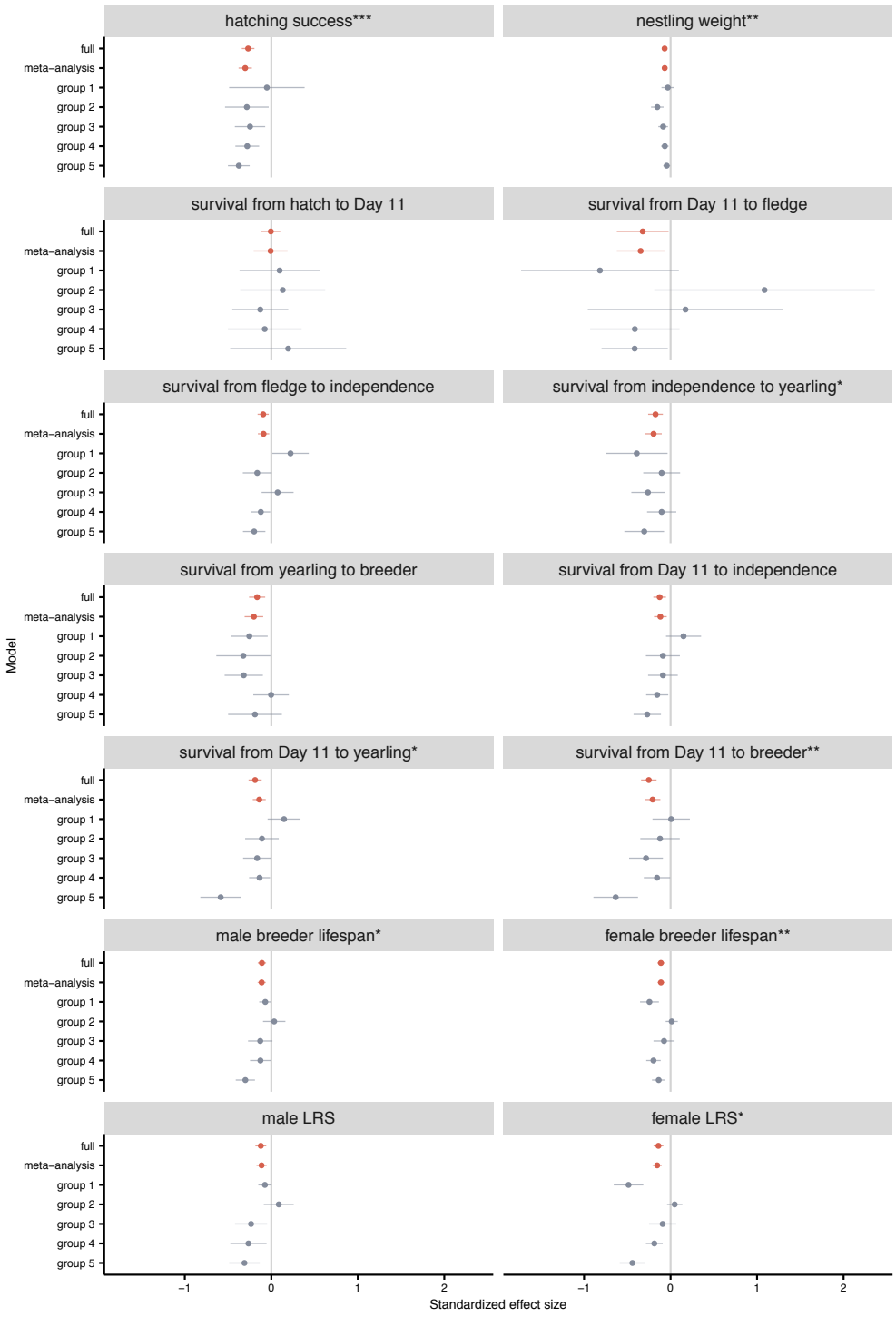


**Figure S3.** Related to Experimental Procedure and Figure 3. Estimated standardized effect sizes of inbreeding for different traits. To test for autocorrelation as a confounder, we split our dataset into five groups, each containing samples from every five years. Estimates are provided for six models: the effect size from the original model using the full dataset, the combined effect size from a meta-analysis of the five subsets, and effect sizes from models using each of the five subsets. Error bars ± SEM. The meta-analysis results are nearly the same as the results from the full dataset (red dots) suggesting autocorrelation is not a confounder; therefore we report values from the full dataset in the main text.

**Table S1.** Related to Figure 2. The coefficient of determination for different models of mean cohort inbreeding levels through time. The first set of models consider the proportion of immigrant-immigrant, immigrant-resident, and resident-resident parents for all individuals with known-status parents and grandparents. The second set of models look at the number of immigrant grandparents for offspring of resident-resident pairs.

| Model | $R^2$ |
|---|---|
| *Parents of all individuals* | |
| (1) constant *p* & constant *F* | -0.007 |
| (2) constant *p* & time-varying *F* | 0.896 |
| (3) time-varying *p* & constant *F* | 0.342 |
| (4) time-varying *p* & time-varying *F* | 1 |
| | |
| *Grandparents of resident-resident birds* | |
| (1) constant *p* & constant *F* | -0.004 |
| (2) constant *p* & time-varying *F* | 0.747 |
| (3) time-varying *p* & constant *F* | 0.347 |
| (4) time-varying *p* & time-varying *F* | 1 |

**Table S2.** Related to Figure 3. Estimated standardized effect sizes for additional fixed effects in the inbreeding depression models. Random effects included in each model were natal year and either pair identity (for hatching success and day 11 weight) or natal nest (for all other models). See Table S3 for sample sizes for each model.

| Variable | β | SE | *p* |
|---|---|---|---|
| *Hatching success* | | | |
| Clutch size | 0.208 | 0.086 | 0.015* |
| Incubation date | -0.135 | 0.079 | 0.087 |
| | | | |
| *Nestling (Day 11) weight* | | | |
| Drought index | -0.193 | 0.041 | $8.3 \times 10^{-5}$*** |
| Number of helpers | 0.084 | 0.027 | 0.002** |
| Brood size | -0.240 | 0.025 | $<1 \times 10^{-12}$*** |
| Sex | 0.105 | 0.039 | 0.007** |
| | | | |
| *Survival from hatch to Day 11* | | | |
| Age of father | 0.244 | 0.131 | 0.063 |
| Pair experience | 0.151 | 0.114 | 0.184 |
| | | | |
| *Survival from Day 11 to fledge* | | | |
| Nestling weight | 2.706 | 0.398 | $9.94 \times 10^{-12}$*** |
| | | | |
| *Survival from fledge to independence* | | | |
| Drought index | -0.194 | 0.109 | 0.075 |
| Rain in previous fall | 0.317 | 0.102 | 0.002** |
| Hatch date | -0.471 | 0.101 | $3.31 \times 10^{-6}$*** |
| Nestling weight | 0.551 | 0.069 | $1.51 \times 10^{-15}$*** |
| Age fledged | 0.257 | 0.073 | 0.0004*** |
| Clutch number | 0.330 | 0.160 | 0.0394* |
| | | | |
| *Survival from independence to yearling* | | | |
| Acorn abundance | 0.399 | 0.183 | 0.030* |
| Juvenile weight | 0.202 | 0.101 | 0.045* |
| Rain in previous fall | 0.261 | 0.184 | 0.157 |
| | | | |
| *Survival from yearling to breeder* | | | |
| Natal territory size | 0.236 | 0.095 | 0.013* |
| Natal territory time since fire | -0.212 | 0.091 | 0.020* |
| Juvenile weight | 0.242 | 0.134 | 0.071 |
| Sex | 0.392 | 0.197 | 0.046* |
| Pair experience | 0.310 | 0.191 | 0.105 |
| | | | |
| ***Combined juvenile traits*** | | | |
| *Survival from Day 11 to independence* | | | |
| Age of father | 0.122 | 0.079 | 0.123 |
| Drought index | -0.445 | 0.161 | 0.006** |
| Rain in previous fall | 0.397 | 0.160 | 0.013* |
| Number of helpers | 0.206 | 0.080 | 0.001** |
| Hatch date | -0.478 | 0.082 | $6.26 \times 10^{-9}$*** |
| Nestling weight | 0.724 | 0.077 | $<1 \times 10^{-12}$*** |
| | | | |
| *Survival from Day 11 to yearling* | | | |
| Drought index | -0.359 | 0.169 | 0.033* |
| Rain in previous fall | 0.513 | 0.161 | 0.001** |
| Natal territory size | 0.143 | 0.070 | 0.041* |
| Number of helpers | 0.141 | 0.069 | 0.040* |
| Hatch date | -0.254 | 0.071 | 0.0004*** |
| Nestling weight | 0.507 | 0.071 | $8.52 \times 10^{-13}$*** |
| | | | |
| *Survival from Day 11 to breeder* | | | |
| Drought index | -0.332 | 0.152 | 0.029* |
| Rain in previous fall | 0.315 | 0.152 | 0.038* |
| Natal territory size | 0.164 | 0.068 | 0.016* |
| Natal territory time since fire | -0.165 | 0.070 | 0.019* |
| Number of helpers | 0.163 | 0.065 | 0.012* |
| Hatch date | -0.188 | 0.071 | 0.008** |
| Nestling weight | 0.460 | 0.075 | $9.9 \times 10^{-10}$*** |
| Sex | 0.206 | 0.124 | 0.096 |

*$p < 0.05$
**$p < 0.01$
***$p < 0.001$

**Table S3.** Related to Figure 3. Estimated standardized effect sizes of inbreeding and sample sizes for different traits. Adult traits were analyzed for each sex separately. Estimates are for individual inbreeding coefficients for all traits except for hatching success and survival from hatch to Day 11, which use proportion IBD-sharing between the parents as a measure of expected inbreeding. Results are provided for both genomic-based inbreeding estimates and pedigree-based inbreeding estimates. The average number of lethal equivalents per individual (with 95% confidence intervals) is provided for survival traits.

| Trait | Sample size | Genomic-based inbreeding | | | Pedigree-based inbreeding | | | Lethal equivalents |
|---|---|---|---|---|---|---|---|---|
| | | β | SE | *p* | β | SE | *p* | |
| *Juvenile traits* | | | | | | | | |
| hatching success | 769 | -0.268 | 0.073 | 0.0002*** | -0.254 | 0.069 | 0.0002*** | 1.697 (0.385, 4.598) |
| nestling (Day 11) weight | 2019 | -0.069 | 0.024 | 0.004** | -0.055 | 0.025 | 0.031* | NA |
| survival from hatch to Day 11 | 804 | -0.006 | 0.106 | 0.959 | -0.044 | 0.106 | 0.679 | 0.032 (-0.450, 2.976) |
| survival from Day 11 to fledge | 2019 | -0.322 | 0.299 | 0.282 | -0.302 | 0.423 | 0.476 | 0.0002 ($-1.581 \times 10^{-6}$,0.661) |
| survival from fledge to independence | 1643 | -0.094 | 0.065 | 0.146 | -0.020 | 0.064 | 0.749 | 2.600 (-0.521, 8.184) |
| survival from independence to yearling | 939 | -0.174 | 0.085 | 0.042* | -0.088 | 0.081 | 0.279 | 3.672 (0.065, 10.780) |
| survival from yearling to breeder | 623 | -0.164 | 0.093 | 0.079 | $-7.7 \times 10^{-5}$ | 0.091 | 0.999 | 5.155 (-0.333, 13.782) |
| *Combined juvenile traits* | | | | | | | | |
| survival from Day 11 to independence | 2019 | -0.126 | 0.073 | 0.083 | -0.046 | 0.159 | 0.726 | 3.838 (-0.327, 10.174) |
| survival from Day 11 to yearling | 2019 | -0.187 | 0.076 | 0.014* | -0.075 | 0.071 | 0.288 | 7.478 (1.215, 14.757) |
| survival from Day 11 to breeder | 2019 | -0.251 | 0.089 | 0.005** | -0.055 | 0.072 | 0.445 | 11.434 (3.135, 20.113) |
| *Adult traits* | | | | | | | | |
| male breeder lifespan | 260 | -0.109 | 0.048 | 0.023* | -0.049 | 0.053 | 0.360 | NA |
| female breeder lifespan | 260 | -0.112 | 0.040 | 0.005** | -0.090 | 0.054 | 0.099 | NA |
| male LRS | 260 | -0.121 | 0.063 | 0.054 | -0.078 | 0.127 | 0.542 | NA |
| female LRS | 260 | -0.140 | 0.057 | 0.014* | -0.089 | 0.058 | 0.128 | NA |

*$p < 0.05$
**$p < 0.01$
***$p < 0.001$

**Supplemental Experimental Procedures**

*SNP discovery and genotyping*

We used genotyping-by-sequencing (GBS) of 103 individuals to discover genome-wide SNPs in the FSJ [S1]. The SNP discovery panel included immigrants and residents from 1978–2008 to help minimize ascertainment bias. SNPs were called using both a custom reference-free pipeline [S1] and a reference-based pipeline. For the reference-based pipeline, demultiplexed and adapter-trimmed reads were aligned to the draft FSJ genome using BWA [S2]. We used Picard tools (http://picard.sourceforge.net) to sort and merge the individual BAM files before indel realignment and variant calling using GATK [S3]. We then designed custom Illumina iSelect BeadChips for 15,416 SNPs. Because the length of flanking sequence required for Illumina iSelect BeadChip assays (50-60 bp on either side) is greater than our GBS read lengths, we could only use SNPs that could be aligned to the FSJ genome. SNPs called using the reference-free pipeline were mapped to the FSJ genome using BWA, and the two sets of SNP calls combined based on their physical location. After thinning to one SNP per 100 bp window, there were 41,853 SNPs. Summary statistics for all SNPs were calculated using custom Perl scripts or VCFtools [S4], and a quantitative score of Mendelian inheritance was assigned using MendelChecker [S1].

We used a number of criteria when designing custom Illumina iSelect BeadChips. First, we filtered out all sites with low mapping quality or read depth (MQ<35, QD<2), high levels of missing data (>8%), excess heterozygosity (>75%), low minor allele frequency (MAF<0.02), or low Mendelian inheritance scores ($M$<-20). We removed SNPs that were fewer than 50 bp from the end of a scaffold or had more than 2 alleles. Flanking sequences for each SNP assay were derived from the draft FSJ genome. We checked for repetitive elements in the flanking sequences using RepeatMasker (http://www.repeatmasker.org) and removed any sites near repetitive elements. The remaining 19,087 SNPs were submitted to Illumina's Assay Design Tool for evaluation. Each SNP was assigned a score that represents the expected success rate of the assay. The final 20k BeadChip design consisted of 17,628 SNPs, each with a minimum score of 0.781 and MAF > 0.0223.

Custom iSelect BeadChips were manufactured by Illumina, and assay design was successful for 15,416 SNPs. Putative chromosomal locations of SNPs were assigned by aligning the FSJ genomic scaffolds to the Zebra Finch genome using standalone BLAST [S5] and picking the best BLAST hit. Given the high degree of synteny among extant bird lineages [S6], we are confident that these SNPs are well distributed across the genome (Figure S1).

DNA samples from different years were mixed in a semi-randomized order on 96-well plates to minimize any batch effects. We genotyped a total of 4,032 samples with the custom BeadChips at Geneseek, Inc. (Lincoln, NE), representing 3,984 unique individuals. For positive controls, 1 individual was genotyped 42 times and 7 individuals were genotyped twice. SNP quality control and correction of inconsistent pedigree relationships were performed in GenomeStudio (Illumina, San Diego, CA), PLINK [S7] and PedCheck [S8]. We removed SNPs with Gentrain score > 0.7 and call rate > 95%, and individuals with call rate > 95%. Reproducibility between duplicate samples was high (>98%). We checked for Mendelian inconsistencies using PLINK and PedCheck [S8]. A very small percentage of nests have extra-pair paternity, and those were removed from subsequent analyses. After correcting any clear pedigree errors, we removed 4 individuals with high Mendelian error rates and any remaining genotypes with Mendelian inconsistencies. To obtain unbiased estimates of genetic diversity and relatedness in this study, we wanted only autosomal SNPs in approximate linkage equilibrium. We excluded 365 SNPs on the Z chromosome because sex-linked SNPs would skew our estimates of mean heterozygosity. We pruned SNPs in high linkage disequilibrium (LD) using the PLINK option --indep 50 5 2. Our final dataset consisted of 7,834 autosomal SNPs in approximate linkage equilibrium in 3,583 individuals.

We checked for an influence of DNA concentration, plate number and location, DNA extraction method and year, and collection year on sample call rate and heterozygosity, and found no strong batch effects. We verified the correctness of our genotype data by checking for Mendelian errors (we found few but all were true pedigree errors) and used a stringent call rate filter (0.95); therefore it is highly unlikely that batch effects will have a significant effect on downstream analyses. There is no significant correlation between DNA concentration and heterozygosity (Spearman's rho = 0.026, $p$ = 0.104). Although our SNP discovery panel included both immigrants and residents from 1978–2008, we sampled more residents than immigrants. It is possible that any observed differences in heterozygosity between immigrants and residents may be due to our ascertainment scheme. However, we compared mean heterozygosity calculated from our BeadChip data to values calculated from the GBS data (after applying standard filters), and found a significant correlation (Spearman's rho = 0.476, $p = 1.383 \times 10^{-5}$). In the sample of 77 individuals with high quality genotypes in both BeadChip and GBS datasets, immigrants did not have significantly different observed heterozygosity from residents using either dataset (Wilcoxon rank sum test, $p$ > 0.1 for both tests).

*Estimation of inbreeding coefficients*

Because pedigree-based inbreeding coefficients only provide an expectation for the mean IBD and are limited by available pedigree information, inbreeding estimates obtained from thousands of genetic markers that provide a measure of realized IBD are more precise [S9]. Any kinship coefficient calculated from the pedigree for a pair containing an immigrant will be zero even if the true kinship coefficient is higher than zero. We calculated pedigree-based inbreeding coefficients for each individual using the R package pedigree [S10] and kinship coefficients for each pair of individuals using the R package kinship2 [S11]. We compared four different genomic estimators of individual inbreeding coefficients: an estimate based on SNP homozygosity implemented in PLINK, an estimate based on the variance of additive genotype values ($F^{I}$ in GCTA), an estimate based on excess homozygosity ($F^{II}$ in GCTA), and an estimate based on the correlation between uniting gametes ($F^{III}$ in GCTA) [S12] (Figure S2). Here, we decided to use $F^{III}$ because it is often one of the more accurate estimators of inbreeding coefficient [S9, 13-15] and in our case it had the highest correlation with pedigree-based inbreeding coefficients, homozygosity, and relatedness of parents.

We tested for inbreeding depression using both pedigree-based estimates of inbreeding and genomic-based estimates of inbreeding (Table S3). Similar to previous studies [S9, 13-15], we find that our genomic estimator of inbreeding coefficients ($F^{III}$) provided much more power for detecting inbreeding depression: we fail to detect inbreeding depression in six of eight fitness-related traits using pedigree-based estimates. Therefore, we use results from genomic estimators of inbreeding coefficients in the main text.

*Modeling temporal variation in inbreeding*

To investigate the impact of decreased immigration on changes in mean inbreeding of the birth cohort over time, we wrote the mean expected inbreeding coefficient of the birth cohort each year ($t$) from 1995 to 2013 as a function of the proportion of immigrant parents and the mean IBD between those pairs:

$$F_{exp}(t) = p_{II}(t)F_{II}(t) + p_{IR}(t)F_{IR}(t) + p_{RR}(t)F_{RR}(t)$$

where $F_{exp}(t)$ is the mean IBD between parents of the birth cohort in year $t$, $p_{II}(t)$, $p_{IR}(t)$, and $p_{RR}(t)$ are the proportion of offspring with immigrant-immigrant, immigrant-resident, and resident-resident parents in year $t$, and $F_{II}(t)$, $F_{IR}(t)$, and $F_{RR}(t)$ are mean IBD between immigrant-immigrant, immigrant-resident, and resident-resident breeding pairs in year $t$, weighted by the number of offspring. Here we used IBD between the parents as a proxy for the expected inbreeding coefficient of the offspring, and we only included offspring with no unknown-status parents or grandparents. We then fitted four models, holding the $p$ terms and/or the $F$ terms constant (we used the mean across all years) or using the observed values for each year:

1. Constant $p$ & constant $F$: $F_{exp}(t) = \overline{p_{II}}\overline{F_{II}} + \overline{p_{IR}}\overline{F_{IR}} + \overline{p_{RR}}\overline{F_{RR}}$
2. Constant $p$ & time-varying $F$: $F_{exp}(t) = \overline{p_{II}}F_{II}(t) + \overline{p_{IR}}F_{IR}(t) + \overline{p_{RR}}F_{RR}(t)$
3. Time-varying $p$ & constant $F$: $F_{exp}(t) = p_{II}(t)\overline{F_{II}} + p_{IR}(t)\overline{F_{IR}} + p_{RR}(t)\overline{F_{RR}}$
4. Time-varying $p$ & time-varying $F$: $F_{exp}(t) = p_{II}(t)F_{II}(t) + p_{IR}(t)F_{IR}(t) + p_{RR}(t)F_{RR}(t)$

We calculated the coefficient of determination for each model as follows:

$$R^2 = 1 - \frac{SS_{residual}}{SS_{total}} = 1 - \frac{\sum_i (y_i - f_i)^2}{\sum_i (y_i - \bar{y})^2}$$

where $y_i$ are the observed values, with associated predicted values $f_i$. We used the coefficient of determination of Model 3 to determine the proportion of the variance in cohort inbreeding explained by temporal variation in the proportion of immigrant parents (Table S1).

We then used the same logic to look at the importance of grandparent immigrant ancestry in explaining the variation in inbreeding observed in offspring of resident-resident pairs in 2002 to 2013 (we dropped before 2002 as sample sizes are too small). In this case, individuals were separated into groups based on the number of immigrant grandparents:

$$F_{RR}(t) = p_0(t)F_0(t) + p_1(t)F_1(t) + p_2(t)F_2(t) + p_3(t)F_3(t) + p_4(t)F_4(t)$$

where $p_i(t)$ is the proportion of offspring with $i$ immigrant grandparents in year $t$ and $F_i(t)$ is the mean IBD between parents of children with $i$ immigrant grandparents in year $t$. We fit the same four models as above and calculated the coefficient of determination for each (Table S1).

*Mixed model covariates*

Fixed effects considered in the inbreeding depression models included characteristics of the individual (sex, nestling and juvenile weight), the natal nest (clutch size, brood size, incubation date, hatch date, age at fledgling, the number of helpers at the territory, pair experience, the ages of the female and male breeders), the natal territory (territory size, time since fire of that territory), and the natal year (acorn abundance, breeding density, drought index, precipitation, and temperature).

Sex of individuals was determined using standard molecular sexing protocols or by behavioral observations in the field. Nestling weight was measured at Day 11, and juvenile weight at Day 85. Not all nestlings were weighed exactly on Day 11; therefore we used a linear regression between weight and age to estimate Day 11 weight for all individuals. The same was done for juvenile weight.

We used the Julian date for incubation and hatch date. Age at fledgling was measured in days. Brood size refers to the number of nestlings that hatched in a given nest. FSJs are cooperative breeders, which means that offspring delay dispersal and help their parents raise future nestlings. We included the number of helpers at a natal territory as a fixed effect. Pair experience was the number of years a given pair has bred together. Breeder age for birds born outside the study tract was estimated as two plus the number of years they were known to breed.

Every territory in the study population was mapped each year and digitized as ArcGIS shapefiles, and detailed habitat and fire history maps were available for the study area, providing fine-scale data on habitat composition and fire history at 3-$m^2$ resolution. We calculated territory size and the average time since last fire for each territory. Acorns are an important food source for FSJs in the winter, when insect abundance is low [S16]. Each year, plant height and the number of acorns and stems were measured for a number of marked oak plants across the study tract. Here our measure of acorn abundance was the total acorn count across all five oak species each year. Annual breeding density was modeled as the inverse of the mean territory size for that year. Daily rainfall in inches, drought index, and temperature data were obtained from the Archbold Biological Station weather station (http://www.archbold-station.org/station/html/datapub/data/data.html). We included mean rainfall in the previous summer (June – August), the previous fall (September – November), and the current breeding season (March – May). The Keetch-Byram drought index is a number from 0 to 800 that reflects soil moisture levels, with 0 indicating no drought and 800 indicating maximum drought. We considered mean drought index in the current breeding season. Because we know that only minimum temperatures affect FSJ phenology, we considered two ways of measuring minimum temperature deviations: the minimum temperature in January – March and the number of degree days below freezing in December – March.

*Temporal autocorrelation*

Given that we are analyzing longitudinal data, it is possible that our data show autocorrelation across years. We used the Acf() and Pacf() functions in the R package forecast [S17] to test for autocorrelation in the residuals of our models. Some of our models showed significant peaks at high time lags. We used a meta-analysis to determine the importance of temporal autocorrelation in our inbreeding depression models. We divided our dataset into five groups, sampling every five years. The mean breeding lifespan of adults is less than five years; therefore this sampling scheme should largely break any temporal autocorrelation caused by the same individuals breeding year after year. We then fit the appropriate model for each trait on these subsets, and found that subsetting our data largely eliminates autocorrelation in the residuals of our models. We calculated a combined effect size using a fixed-effects model with inverse-variance weights using the R package metaphor [S18]. The results from the meta-analysis are not significantly different from the results of our original models (Figure S3). Therefore, we report the results from the original models in the main text.

*Estimating lethal equivalents*

We used an approach similar to that described in [S19] to estimate the number of lethal equivalents per individual and their 95% confidence intervals for our survival traits. Briefly, we used the final model of each trait to predict survival probabilities when $f = 0$ (outbred) and $f = 0.25$ (inbred), using the mean for all other fixed effects. Instead of using pedigree-based inbreeding coefficients, we used the genomic-based estimator of inbreeding coefficient that corresponds to the pedigree-based inbreeding coefficient of 0 and 0.25 ($F^{III}$ = -0.065 and 0.324, respectively). For hatching success and survival from hatch to Day 11, which used proportion IBD-sharing between the parents as a measure of expected inbreeding, we substituted IBD = 0 for $f = 0$ and IBD = 0.5 for $f = 0.25$. Note

that the original models were fit with standardized fixed effects, so we used standardized values when predicting survival. We used the invlogit function in the R package arm [S20] to back-transform our estimated probabilities from a logit scale. We substituted these values in the equation from [S21] to calculate the number of lethal equivalents:

$$B = -\ln (S_f/S_0)/f$$

where $S_f$ is the survival probability at inbreeding level $f$ (conventionally set at 0.25) and $S_0$ is the survival probability at $f = 0$. To calculate the 95% confidence interval, we repeated the above process, substituting in the lower and upper bounds for each parameter estimate.

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