Master's thesis

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

Nitya Shrestha

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Master's thesis in Natural Resource Management
Supervisor: Henrik Jensen
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Abstract

Inbreeding and inbreeding depression have been a major challenge for conserving small and isolated populations. The viability of endangered species has been threatened by inbreeding depression; thus, it has been an indispensable part of conservation genetics. There are many studies on inbreeding depression in the laboratory but studying it is quite challenging in the wild or natural population. However, some recent studies incorporating extensive longitudinal datasets have made it possible to study inbreeding depression even in natural populations. For studying inbreeding depression in the wild, especially for vulnerable and endangered species, gathering data about pedigree and individual life history is not feasible. So, there is a need for an easier alternative approach to detect inbreeding depression in the wild. Thus, this study demonstrates an alternative approach for detecting inbreeding depression without needing pedigree and individuals' life histories. I used data on an insular house sparrow, *Passer domesticus,* metapopulation on the Helgeland coast in northern Norway collected from 10 different islands, and years 2007 to 2014. This study examined the change in inbreeding levels across nestling, juvenile, and adult stages, and with age within the nestling and adult stages in the metapopulation. The result showed a significant decrease in inbreeding levels from nestling to the juvenile and adult stages but insignificant from juvenile to adult. This decrease in inbreeding levels across stages in this metapopulation gives evidence of the selective disappearance of inbred individuals caused by inbreeding depression in survival. Also, the effect of inbreeding in house sparrows varied with sex. Within nestling and adult stages, males and females have higher selective disappearances, respectively.

Thus, this method can be used as an alternative approach for studying inbreeding depression in species for which collecting fitness data and pedigree can be difficult, especially for endangered species. This method can be particularly used in detecting the effectiveness of conservation activities by tracking the inbreeding level for a longer time and at different stages. Also, the highly affected stages can be facilitated to cope with the negative effect of inbreeding by providing extensive care or modifying habitat especially if the population is endangered or about to become extinct.

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1. Introduction

Global wildlife populations have fallen by an average of 69 % from 1970 to 2018 (WWF, 2022). Habitat loss and fragmentation are reported as major causes as they are major threats to biodiversity (Brooks et al., 2002; Hanski, 2005; Schlaepfer et al., 2018). It directly fragments, isolates, and decreases the size of populations and can lead to reduced gene flow, shrinking genetic variability within remnant populations through genetic drift and inbreeding (Mullu, 2016). Fragmented populations and increased isolation favor genetic drift and reduced gene flow. Genetic drift refers to random fluctuation in allele frequency in a population over generations caused by random chance rather than by natural selection. Genetic drift can cause complete loss of genetic variants from the population and fixation of certain alleles, even if harmful, decreasing fitness along with genetic diversity. Reduced population size might lead to inbreeding due to the low availability of genetically unrelated mates. Additionally, isolated populations generally depend on immigration for their persistence (Robinson et al., 1995), but decreased connectivity reduces immigration and gene flow, increasing the chance of close relatives mating (inbreeding) and ultimately reducing the population viability (Leimu et al., 2006).

Inbreeding is common in small and isolated populations. Inbreeding refers to mating between genetically close relatives resulting in increased homozygosity in the offspring because they are more likely to inherit the same allele from both parents (Frankham et al., 2002). The individual inbreeding level is estimated by calculating the inbreeding coefficient F. It is the probability that both alleles at a locus in an individual's genome are identical by descent (IBD) (Nelson & Crone, 1999). Traditionally the inbreeding coefficient (F) was mostly estimated from a known pedigree using a method developed by Wright a century ago (Wright, 1922). In the pedigree-based estimator, the expected level of inbreeding of an individual depends on the extent of ancestry in the pedigree that the parents of that individual share (Keller, 2002). However, building the pedigree by tracking the reproduction and survival of a large proportion of individuals in a population over several generations can be difficult, especially in wild populations (Ballou, 1983; Hedrick & Kalinowski, 2000; Balloux et al., 2004; Jensen et al.,

2007; Billing et al., 2012). Pedigree-based F estimators could be unreliable due to problems with accurate identification of ancestry (Pemberton, 2008), are also often costly, and time-consuming, therefore may not be feasible in many study systems.

Recent studies found that a marker-based estimator of the inbreeding coefficient is more precise and less biased than pedigree-based estimation as it overcomes the potential bias from incomplete, inaccurate pedigree (Keller et al., 2011; Kardos et al., 2015). Modern genetic technologies utilize genomic data and markers to estimate inbreeding coefficients. F could be estimated accurately using high-density single nucleotide polymorphism (SNPs) as genetic markers (Allendorf et al., 2010) without the need for pedigree data. F_{GRM} and F_{ROH} are two types of inbreeding coefficients based on genome-wide SNPs (Mastrangelo et al., 2016). F_{GRM} estimates the inbreeding coefficient from the genomic relationship matrix (GRM) (VanRaden et al., 2011). GRM is a square matrix used in genetic analysis to capture the genetic similarity between pairs of individuals based on genotypes at genetic markers. F_{ROH} is based on the detection of runs of homozygosity (ROH) (Gibson et al., 2006). ROH are uninterrupted stretches of homozygous genotypes that exist in an individual because of parents passing on identical haplotypes to their progeny. As recombination event disrupts lengthy chromosomal regions, long stretches of ROH refer to recent inbreeding while short stretches indicate ancient inbreeding events. Thus, F_{ROH} is considered the most powerful estimator of the inbreeding levels of individuals (McQuillan et al., 2008; Keller et al., 2011).

Inbreeding levels across individuals could be affected by multiple factors, out of which population demography is one. A large population favors better selection against deleterious alleles and a low probability of mating with close relatives reducing inbreeding (Demontis et al., 2009; Hedrick & Garcia-Dorado, 2016). However, mating between close relatives is more common in small populations due to less availability of mating choices, resulting in high inbreeding levels and high proportions of homozygous loci. This exposes deleterious recessive alleles/mutations, which may be removed from the population through natural selection over time also known as purging (Lande & Schemske, 1985; Hedrick & Garcia-Dorado, 2016).

However, natural populations are rarely at mutation-selection drift equilibrium making the purging effect controversial (Charlesworth, 2018). For example, purging can be counteracted

by dispersal and gene flow. Immigration can provide both good and bad effects. Dispersal may create a heterosis effect and increase the fitness of offspring produced from parents from different populations by masking the effects of deleterious recessive alleles through a reduction in the homozygosity of such alleles (Dobzhansky, 1950).

The negative effect of inbreeding in the reduction of fitness is referred to as inbreeding depression (Charlesworth & Willis, 2009). Inbreeding reduces fitness through an increase in the occurrence of homozygous loci (Charlesworth & Willis, 2009). There are two hypotheses explaining how inbreeding affects the fitness of individuals (Charlesworth & Charlesworth, 1999; Keller, 2002). According to the overdominance hypothesis, being heterozygous at a gene locus has more fitness advantages than being homozygous for either allele at that locus, thus increased homozygosity from inbreeding leads to decreased fitness. On the other hand, the partial dominance hypothesis states that reduced fitness from inbreeding is due to increased homozygosity that leads to increased expression of recessive or partially recessive deleterious alleles. Indicating that dominant alleles are less likely to mask the negative effect of recessive deleterious alleles. Regardless of the underlying mechanism, inbreeding depression can also be represented as inbreeding load which means the negative consequence of inbreeding in fitness or an increase in the frequency of harmful traits in a population.

Inbreeding has negative effects at both individual and population levels (Keller, 2002). Inbreeding reduces the fitness of organisms through its influence on fitness-related traits (Charlesworth & Willis, 2009) including life history and morphological traits (Keller, 2002). However, inbreeding depression is usually more severe in life-history traits than morphological traits (Roff, 1998; DeRose & Roff, 1999; Wright et al., 2008). The magnitude of inbreeding depression might therefore differ on different fitness traits and life history stages (Grueber et al., 2010; Harrisson et al., 2019; Huisman et al., 2016). Also, inbreeding depression on survival may be most strong in early life stages like hatching and nestling compared to juvenile and adult stages (Sittmann et al., 1966; Keller, 1998; Hemmings et al., 2012). For example, in a study of a wild population of song sparrow, *Melospiza melodia*, from British Columbia, an inbreeding coefficient of 0.25 was reported to reduce the survival from egg to breeding by 49%, adult survival rate by 24% and reproductive success of female throughout the life by 48% (Keller, 1998). Furthermore, a study on insular house sparrows in Norway demonstrated a

decreased likelihood of fledgling recruitment because of inbreeding (Jensen et al., 2007). Another study in the same house sparrow metapopulation discovered that inbreeding reduced the reproductive output and adult annual survival, and consequently LRS (number of recruits produced over an individual's lifespan) (Niskanen et al., 2020). Also, the study of endangered species, such as the red-cockaded woodpecker, *Picoides borealis* from southeastern USA found that inbreeding reduces the hatching rate, fledging success, and recruitment to the breeding population (Daniels & Walters, 2000). Inbreeding depression can seriously affect small and isolated populations and even lead to extinction through a reduction in survival and reproduction success (Caughley, 1994; Saccheri et al., 1998). For example, a study of the Glanville fritillary butterfly, *Melitaea cinxia* in Finland found that inbreeding increased the extinction risk by affecting its larval survival, adult survival, and hatching success (Saccheri et al., 1998).

Also, the levels and effects of inbreeding on fitness may differ due to population size, environmental conditions, and interaction between genotype and environment (Hedrick & Kalinowski, 2000; Armbruster & Reed, 2005). The study on the effects of inbreeding on individual fitness in the wild, where environmental variation interacts with the expression of deleterious recessive alleles, is crucial for understanding the importance of inbreeding in conservation, wildlife management, and evolutionary ecology (Frankham, 2022). As inbreeding reduces the adaptation capacity of a population to a changing environment (Jump et al., 2009; Manel & Holderegger, 2013), inbreeding is also expected to be detrimental to population viability in the long term under environmental change (Leimu et al., 2006). Thus, inbreeding may increase the probability of local population extinction or even extinction of species (Mullu, 2016).

Estimating the strength of inbreeding depression is vital to conservation and evolutionary biology (Hedrick & Kalinowski, 2000; Taylor et al., 2010) as a decrease in fitness by inbreeding affects the population viability and several evolutionary and ecological processes (Keller, 2002). However, most of the studies are about inbreeding depression in early developmental or juvenile stage (Daniels & Walters, 2000; Keller, 2002; Kruuk et al., 2002). Though there are also other studies, about the effect of inbreeding on adult life-history components like reproductive success (Keller, 1998), there is still a need for studies that

examine the effect of inbreeding across all life stages of an organism (Baalsrud et al., 2014). In small and endangered populations, detecting inbreeding depression can be difficult if it is not very strong due to a low level of inbreeding load because of low statistical power and difficulty in measuring every kind of fitness component at every life stage (Hedrick & Kalinowski, 2000). Studying changes in the inbreeding coefficient can be easier than inbreeding depression as it does not need to focus on individual fitness measurements, which generally require more resources. Examining the change in inbreeding levels across the life stages helps to determine if there is any selective disappearance of inbred individuals. If there is a decrease in inbreeding levels in later life stages compared to earlier stages, this would indicate the negative effects of inbreeding on survival. Therefore, studying changes in inbreeding levels is an easier way to detect indirect evidence of inbreeding depression. For the conservation and management of wild populations, it would be practical to see a change in inbreeding coefficients over time as it would provide insight into the genetic health of populations and could help predict the population viability.

This study aims to determine how the inbreeding level changes within and across different life stages, particularly from nestling, juvenile, and adult stages, and within nestlings and adults in an insular house sparrow metapopulation. I predict that the average level of inbreeding or inbreeding coefficient decreases along with an increase in age or proceeding of life stages because the inbred individuals will probably die in the early stage because of selection against inbred individuals. I used data from cohorts (birth years) from 2007 to 2014 and from 10 different islands in northern Norway. 6215 unique individuals were tracked across various life stages like nestlings, fledged juveniles, and adults. To achieve the goals of this study, I used: a) The genomic inbreeding coefficient estimator F_{ROH} as the individual inbreeding coefficient, and b) estimates of F_{ROH} for individuals at nestling, juvenile, and adult stages, and nestlings and adults at different ages in days and years, respectively.

This study will help in understanding the inbreeding dynamics or changes in inbreeding levels in this metapopulation. It will give an idea about the developmental stage with the highest inbreeding load, i.e. the stage with the greatest influence of inbreeding on fitness and survival. Knowing the inbreeding dynamics in the population can help in interventions in conserving and managing a population.

2. Methods

2.1 Study area

This study was conducted within a house sparrow metapopulation study system with 18 different islands in an archipelago off the coast of Helgeland in northern Norway (66° N, 13° E; Figure 1).

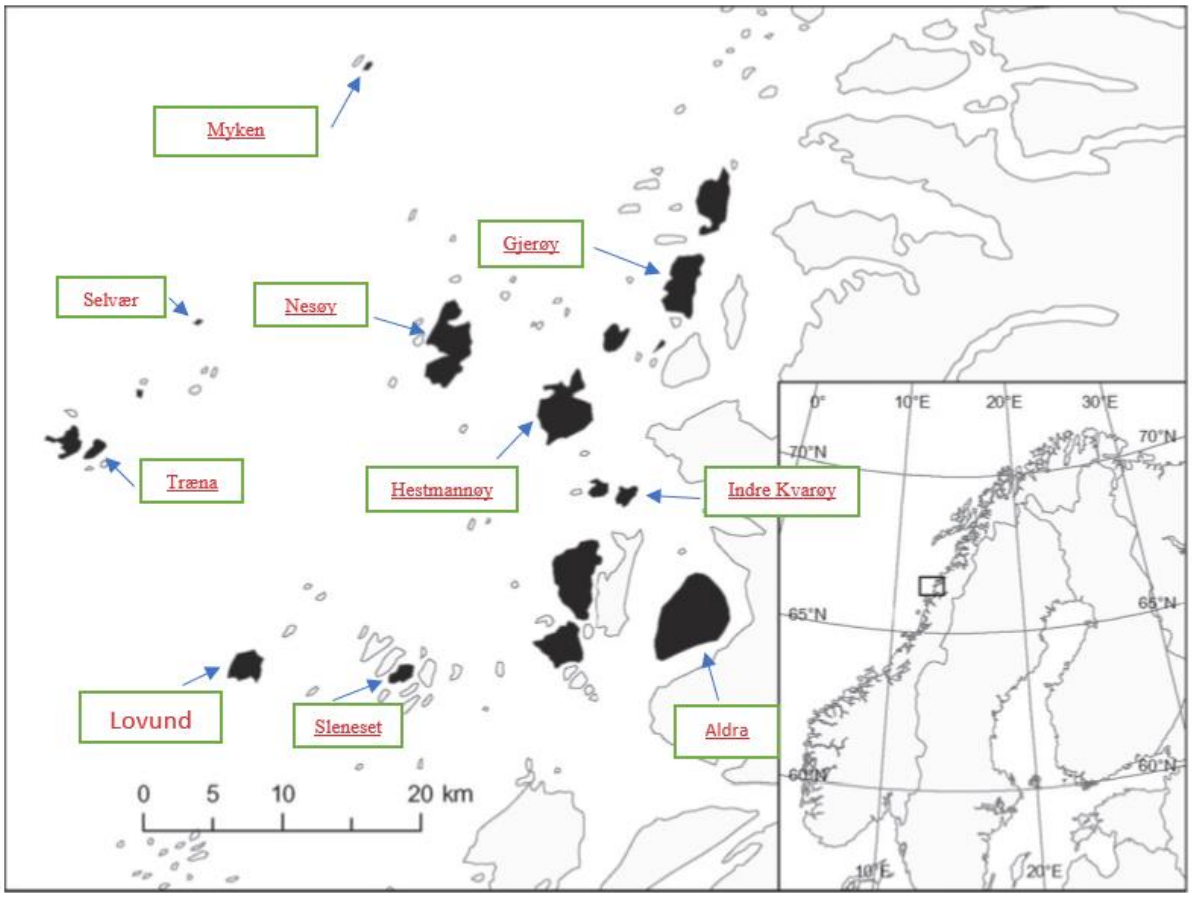

Figure 1:18 islands (represented in black) along the coast of northern Norway with a long-term study of house sparrow populations (Baalsrud et al., 2014). Islands with names in boxes are islands used in this study.

Although the long-term study has been going on since 1993, my study was based on house sparrow data collected over 8 years (2007 to 2014) from 10 of the islands in the system, namely: Aldra, Gjerøy, Hestmannøy, Indre Kvarøy, Lovund, Myken, Nesøy, Selvær, Sleneset, and Træna (Baalsrud et al., 2014).

The study system consists of two habitat types: farm and non-farm islands (Ranke et al., 2021; Saatoglu et al., 2021). In farm islands, sparrows mostly live in dairy farms and have access to cow feed as nests are mostly made inside barns. On non-farm islands, sparrows nest outdoors in nest boxes and around human settlements, and there is a larger variation in food availability compared to farmed islands as they often depend on bird feeders (Pärn et al., 2012). Here, farm islands are Aldra, Gjerøy, Hestmannøy, Indre Kvarøly, and Nesøy and non-farm islands are Lovund, Myken, Selvær, Sleneset and Træna.

2.2 About the Species

The house sparrow, *Passer domesticus* is a common bird from the family Passeridae, and widely distributed across the world being native to a wide range extending from the British Isles through northern Scandinavia, Europe (except Italy), and northern Asia to the Pacific coast (Anderson, 2006).

This bird is approximately 14-16 cm long and is sexually dimorphic with males having brighter plumage with white wing bars and black chest badge while females are plainer. They are omnivorous and feed on insects, seeds, or animal feed scraps from farms or barns. This species is mostly monogamous during the breeding season whereas, some cases of extra-pair paternity have also been recorded (Cordero et al., 1999; Girndt et al., 2018; Vaclav, 2003). In this study area, the breeding season lasts from early May to the middle of August (Ringsby et al., 1998). Each breeding pair has one to three clutches with an average of 5 to 6 eggs (Ringsby et al., 2002; Husby et al., 2006; Jensen et al., 2007). Both parents incubate for ca. 11 days, feed the nestlings for ca. 14 days, and fledged juveniles for around 2 weeks after leaving the nest (Anderson, 2006).

2.3 Collection of samples

Throughout the breeding season, nests were searched for in natural cavities inside barns and nest boxes on the islands. When a new nest was found, it was visited 2-3 times during the incubation and nestling stages. Hatching day was recorded directly or indirectly counting days from the age of nestlings at first visit after hatching. Once eggs were hatched, most nestlings were ringed between 8-12 days old, however, some nestlings were also sampled from 4-7 days old, especially in Gjerøy and Hestmannøy, and few from 13 to 14 days old. Different morphological measurements like body weight, tarsus length, wing length were taken, and each nestling was marked with a numbered aluminum ring and a unique combination of three colored plastic rings (Jensen et al., 2007; Ringsby et al., 1998).

Lastly, a small blood sample (ca. 25 μL) was taken from the nestlings' brachial vein. Similarly, a small blood sample was also taken from fledged juveniles and adults captured by mist-netting, which were also ringed with a unique combination of three colored plastic rings and a numbered aluminum ring, if not ringed from before.

Most of the data for juveniles and adults came through re-captures by mist-netting and observation. Re-capture and re-sighting of birds identified by their unique combination of colored rings enabled to recording of the bird's sex (if not known from before) and which birds were alive at each stage and age and helped in figuring out their stage and age through previous records. A bird was defined as recruited into the adult population if captured or observed in years after its hatching year.

Blood samples from 6215 unique individuals that were tracked across different life stages like nestlings, fledged juveniles, and adults of up to 7 years old were used for DNA genotyping to generate genetic data. The individuals were genotyped using custom 70K house sparrow Axiom Single Nucleotide Polymorphisms (SNPs) arrays (Niskanen et al., 2020).

2.4 Inbreeding coefficient estimation

Genomic inbreeding coefficients F_{ROH} (inbreeding coefficient for runs of homozygosity, ROH) were calculated using PLINK v1.9 (Chang et al., 2015). F_{ROH} is based on runs of homozygosity that result from individuals inheriting identical segments of chromosomes from each parent, due to the parents sharing a common ancestor from which these identical segments originate.

Initially, genomic data were stored in binary format which contained information about SNPs. They were converted into an accessible format to analyze in PLINK. To use as a reference, for detecting homozygous segments in the real dataset, a simulated individual was created with an assumption of complete homozygosity across all SNP loci and added to the original data set.

For ROH detection with PLINK, certain parameters were specified which included a scanning window size of 50 SNPs, minimum length of 2.5 million base pairs (Mb) ROH segment, maximum of 1 heterozygous SNP, up to 5 missing SNPs per window, and minimum density of SNPs being at least 1 SNP per 20 Kb and a maximum of 500Kb between consecutive SNPs. PLINK scanned the genome with these criteria and marked it as an ROH segment where the criteria were met. Further, these detected ROH segments were analyzed in R. The maximum length of ROH from the simulated homozygous individual was used as a reference for the calculation of F_{ROH} of individuals. Thus, F_{ROH} was computed as a proportion of the ROH of individuals to the maximum ROH observed for simulated homozygous individuals. Thus, individual F_{ROH} provides insight into the extent of homozygosity and potential recent inbreeding within a population. Since a minimum length of 2.5 Mb for ROH was used, it covers inbreeding due to shared ancestors of an individual's parents up to approximately 10 generations back. Individuals who are slightly inbred due to parents sharing very distant relatives will have shorter ROH and low F_{ROH} compared to those sharing more recent ancestors. As F_{ROH} indicates a proportion of the genome, its value can vary between 0-1, and offspring of, for example, full-sib mating are expected to have $F_{ROH} \sim 0.25$.

2.5 Statistical analysis

2.5.1 For nestlings

To determine if inbreeding level changes with nestling age, 4785 unique nestlings with 5839 different observations resulting from some repeated individuals across different ages were used. Nestlings ranging from 4 to 14 days old from 10 islands and years 2007 to 2014 were used in the analysis (Table 1). However, some islands have few observations while others have comparatively more observations.

Table 1 Distribution of number of nestlings at each age on different islands in the house sparrow study system.

Islands Age in days	Nesøy	Myken	Trænа	Selvær	Gjerøy	Hest-mannøy	Indre Kvarøy	Lovund	Slenes et	Aldra	Total
4	12	6	5	7	41	26	8	4	10	9	128
5	44	21	43	41	162	235	39	22	24	18	649
6	29	19	23	10	128	181	29	15	10	15	459
7	37	24	24	38	108	135	58	5	24	22	475
8	56	35	43	37	55	80	75	29	22	11	443
9	35	35	136	116	151	120	56	30	102	25	806
10	27	32	155	166	148	174	84	47	58	4	895
11	43	25	178	128	144	242	86	54	77	45	1022
12	43	25	121	89	84	106	40	52	64	12	636
13	11	18	24	30	29	42	16	21	34	7	232
14	3	1	7	14	21	6	4	13	22	3	94
Total	340	241	759	676	1071	1347	495	292	447	171	5839

Linear mixed effect models with and without the quadratic effect of nestling age were fitted. The model with a quadratic effect was fitted with an expectation that the change in inbreeding level with age might not be linear as the decrease in inbreeding level might be high initially and slow down when nestlings grow older, showing a curvilinear effect. Also, a linear mixed effect model without a quadratic term (i.e. only a linear term) of age was fitted with the expectation that the inbreeding coefficient decreases linearly with an increase in age. Both models were fitted with F_{ROH} as a response variable, nestling age and sex as fixed predictors (as covariate and factor, respectively) including an interaction between nestling age and sex,

and year and island as random factors with random intercept. Both models are fitted with a perm. lmer function from the "permutes" package (Voeten, 2023) because residuals are not normally distributed thus, the p-values from the regular linear models may be unreliable.

2.5.2 For stages

For analyzing the change in inbreeding level between different life stages particularly nestling, juvenile, and adult, data from 10 different islands and from 2007 to 2014 further restricted to cohorts 2007 to 2014 were used (see Appendix 1). I used the data from 2007 to 2014 because only these years had all nestlings genotyped. The same individuals could appear multiple times in data at different ages or stages and unique individuals within the same stages were used because I was assessing whether there was a selective disappearance of inbred individuals from a random sample of a finite population. Out of the total data of 9514 records, there were 4785 nestling records, 3001 juvenile records, and 1728 adult records, where most individuals were repeated across different stages but unique within each stage (see Appendix 1These data were unevenly distributed over islands (see Appendix 1 Distribution of sampled data in different stages and islands.). Since the response variable i.e. inbreeding coefficient does not follow a certain distribution and residuals are not normally distributed, I did a permutation model using "perm. lmer" function from package "permutes" (Voeten, 2023). F_{ROH} was the response variable and stage was the main predictor variable. Sex and its interaction with the stage were also included in the model to see if there was a difference in the level of inbreeding between the two sexes and in different stages. However, the final model excluded both sex and interaction because any difference was not evident. As inbreeding seems to vary across islands and cohorts, these variables were also included as random factors. Cohort (birth year) was chosen instead of capture year because cohort might have more impact on inbreeding level because of population size variation. But note that in this study, the year (i.e. sampling year) also gave the same results.

To examine how inbreeding level changes across different life stages, I fitted a linear mixed effect model with the permutation method to find the effect size i.e. change in inbreeding coefficient between these three stages. At first, I fitted a model with the stage as a predictor

with categories ordered nestling, juvenile, and adult. This gives the contrast between nestling and juvenile and nestling and adults. To find a contrast between juvenile to adult, I fitted the same model with juvenile ordered as the first stage.

2.5.3 For Adults

To analyze how inbreeding level changes with age within adults, 2767 observations from 1728 unique adults ranging from ages 1 to 7 years old were used, in which individuals within the same age were unique but could be repeated in later ages if they survived and were sampled. For this analysis also, data from 10 different islands and from cohorts 2007 to 2014 were used (see Appendix 2). First, a linear mixed effect model with the linear and quadratic effects of age was fitted with F_{ROH} as a response variable and adult age, and sex as the predictor variables (as covariate and factor, respectively) along with their interaction, including both island and cohort as random factors with random intercepts (see Appendix 4). The quadratic effect of age was considered with assumptions that at the initial stage, there could be a rapid decrease in the inbreeding level, after a few years, there could be almost no change i.e. the inbreeding coefficient may not change at the same rate throughout adult's age. Also, a linear mixed-effect model without a quadratic effect (i.e. only a linear effect) of age was fitted (see Appendix 5). However, through visual interpretation, it was found that Aldra was different from the other islands (see Appendix 6), which might be due to a quite different population history (Baalsrud et al., 2014; Billing et al., 2012) and I predicted inclusion of data from this special island might affect the results. Therefore, both analyses with and without quadratic effect were done excluding the data from Aldra to test the significance of its effects, with a total of 2668 observations from 1667 unique individuals, but the quadratic term was then not significant anymore (Appendix 7). Finally, a linear mixed effect model was finalized and fitted including age, sex, and their interaction along with random factors, with data excluding Aldra.

2.5.4 General statistics

Upon creating a histogram (see Figure 2) and density curve (see Appendix 8) for F_{ROH}, it was not normally distributed and seemed to instead follow a gamma distribution. So, the

generalized linear model (glms) and generalized mixed effect models (glmer) were first fitted specifying the gamma family and log link. It was found that this model did not fit well and did not meet the model assumptions, after checking the model diagnostic (see Appendix 9). Then, I tried different transformations of F_{ROH} like square root, cubic root, quartic root, log transformation, inverse transformation, etc. Transformed data were fitted in the model but the model diagnostics were not improved.

The next step involved understanding the distribution of data. I used the descdist function from R package "fitdistrplus" to map distribution which showed beta distribution (Muller et al., 2023) (see Appendix 10), so I fitted a zero-inflated beta model as data from package "glmmTMB" (Brooks et al., 2024) because 15% of the data has 0 values (see Appendix 11). I used the R "DHARMa" package to check the model diagnostics (Hartig, 2022) (see Appendix 12). It was found that this model was also not fitted properly. Different distributions like gamma, lognormal, Weibull, etc. which have right tails were fitted to data, to examine if my data fitted any distribution through function fitdist from R package "fitdistrplus" (Muller et al., 2023). However, the plot was not good for any distribution. So, another approach i.e. goodness of fit test called the Anderson-Darling test was done for each distribution, but this test also could not specify any appropriate distribution. The detailed process of examining the distribution of data is given in Appendix **19**. Thus, this data follows a complex structure, which does not seem to correspond with any more or less common type of statistical model. Therefore, I opted for the permutation test because p-values derived from the permutation test are robust to violations of assumptions of normality. I did a permutation test with function perm.lmer from package "permutes". For each test, I did a permutation of 10,000 times i.e. data were shuffled and reshuffled 10,000 times in each model.

Since it is not directly possible to calculate the confidence interval from the model fitted by permutation, I fitted linear mixed effect models (LMM) using the lmer function from the "lme4" package and then used function confint to estimate the 95% confidence intervals of the parameter estimates. Note that the permutation models and equivalent LMM gave the same parameter estimates but differed in p-values because the correct error distribution could not be identified for the LMM. Thus, this study relies on parameter estimates and significance tests from permutation models, but confidence intervals and standard errors of parameter estimates

from LMM, meaning that the reader must be cautious when interpreting the confidence intervals.

The Statistical analyses were done in R version 4.3.3. Data manipulation was done through “tidyverse”. For permutation testing, the package “permutes” was used, and linear mixed effect models were fitted using the “lme4” package. The plots and visualization were created from “ggplots” and “sjPlot.”

3. Results

Out of a total sample of 9514 records of 6215 unique individuals at different stages, 84.6 % of sampled individuals are inbred i.e. had F_{ROH} >0 (Figure 2, Appendix 11). The overall mean and median were 0.0264 and 0.0164 respectively, with a range of 0 to 0.4190 (Appendix 14). The overall distributions of mean inbreeding levels in different life stages, islands, and years/cohorts are shown in Appendix 15,Appendix 16, and Appendix 17.

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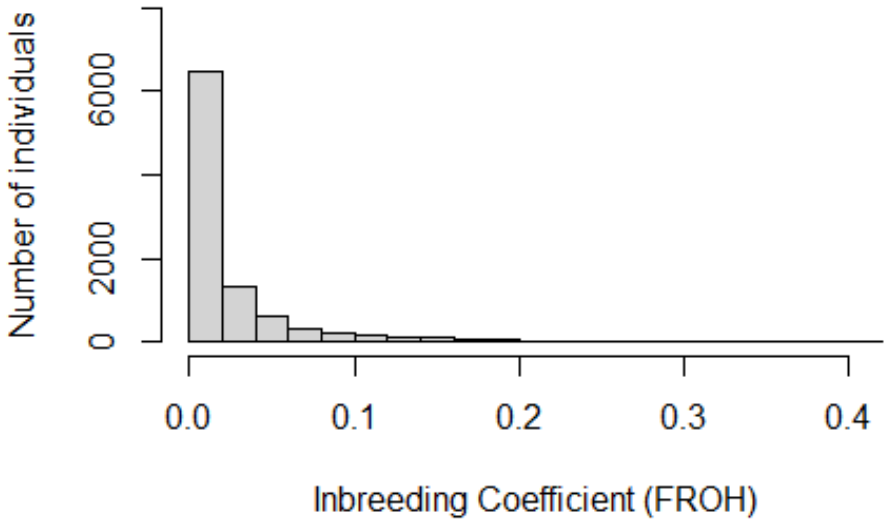


Figure 2. Frequency distribution of inbreeding coefficient of individuals from all stages (nestlings, juveniles, and adults) in house sparrows (n=9514 records, and n= 6215 unique individuals).

3.1 Change in inbreeding level within nestlings

The linear mixed effect model testing for changes in F_{ROH} within the nestling stage was fitted through permutation with nestling age (covariate), sex (factor), and their interaction as fixed predictors including island and year as random factors. The results of this model are shown in Table 2.

The results showed, that initially, males have a slightly higher mean inbreeding coefficient compared to females. The significant interaction term provides evidence of a decrease in the inbreeding coefficient across nestling ages in males, however, the decrease was not significant for females. The result is represented in Figure 3.

Table 2. Parameter estimates from the best model testing for a change in inbreeding levels with age within nestlings in house sparrows.

		Confidence interval			
Parameter	Estimate	2.50%	95%	Std. Error	Pr(>|t|)
Intercept	0.03642	0.02180	0.05108	0.00729	NA
Nestling age	-0.00004	0.00073	0.00064	0.00035	0.4231
Sex(M)	0.00352	0.00532	0.01238	0.00452	0.0014***
Nestling age: Sex(M)	-0.00047	0.00141	0.00047	0.00048	0.0000***

Note: Parameter estimates, and p-values are from a permutation test and confidence interval and standard error from basic linear mixed effect models, so CI and SE are not reliable.

In addition, result from the quadratic model fitted by adding a quadratic term for nestling age in the previous linear mixed effect model was unexpected (Appendix 18). It showed that the

inbreeding level increased initially with nestling age, reached a maximum at around 7 to 8 days of age, and then decreased. The initial increase in inbreeding level was unexpected and does not seem to have a biological reason.

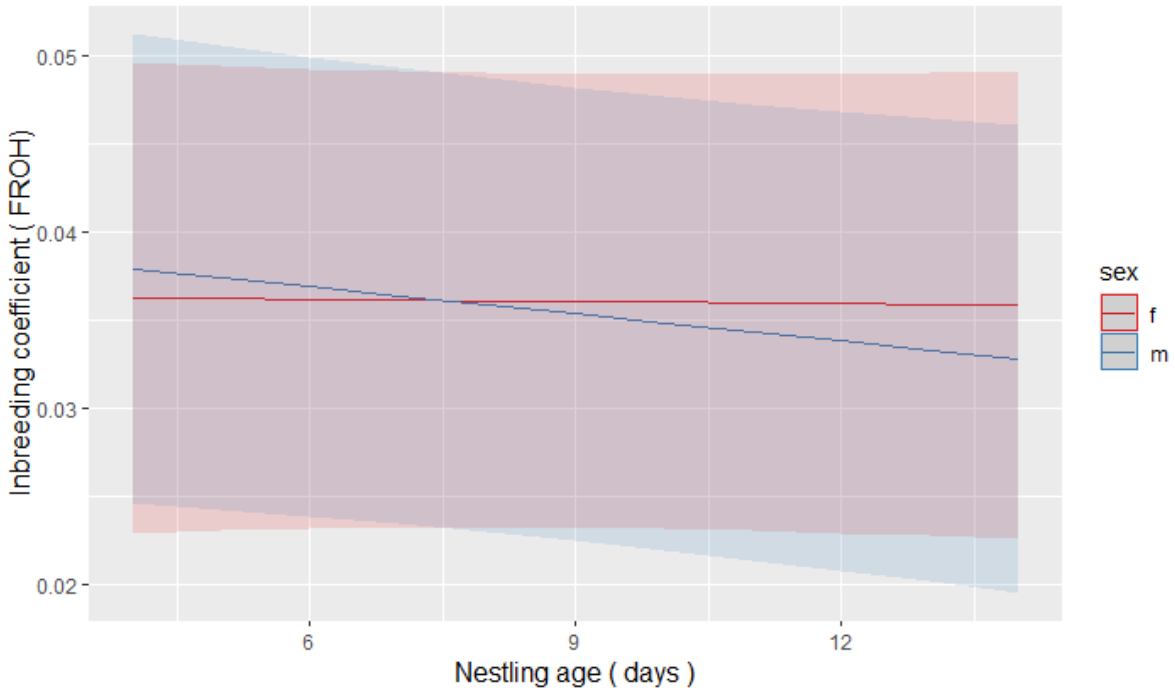


Figure 3. Predicted changes in inbreeding levels with age within female (in red) and male (in blue) house sparrow nestlings.

Note: Confidence intervals are unreliable as they are based on a linear model and not permutation tests.

3.2 Change in inbreeding level between stages

To test how inbreeding levels differed between the three stages, i.e. nestlings, juveniles, and adults, two different linear mixed effect models through permutation tests were fitted, with two different stages as intercept, respectively. First, the model was fitted with the nestling stage as the intercept, highlighting the difference between nestlings and juveniles and between nestlings as adults as shown in Table 3 a).

There was a significant decrease in the inbreeding coefficient from the nestling stage to the juvenile stage of 0.0023. Similarly, there was also a significant decrease in inbreeding level between nestlings and adults of 0.0035. Furthermore, there was a slight overall trend that inbreeding was lower in males than females, which seemed marginally significant.

To estimate the difference in inbreeding levels between juveniles and adults, another model was fitted with the same variables but with the juvenile stage as the intercept. The results are shown in Table 3 b).

There was a trend that the inbreeding coefficient decreased from the juvenile to the adult stage, but it was not significant. Similarly, as in the first model, the second model also found a significant difference in inbreeding levels between juveniles and nestlings. Again, males seemed to have a lower inbreeding coefficient than females, with marginal significance.

Table 3: Parameter estimates from models testing for changes in inbreeding levels between different stages (nestlings, juveniles, and adults) in house sparrows. The two models presented are equivalent, except that model a) had the nestling stage as intercept, whereas model b) had the juvenile stage as intercept.

Parameter	Estimate	Confidence interval		Std. Error	Pr(>|t|)
		2.50%	95%		
a)					
Intercept (Nestling)	0.03542	0.0240	0.0469	0.0056	NA
Stage (Juvenile)	-0.00231	-0.0043	-0.0003	0.0010	0.0057***
Stage (Adult)	-0.00352	-0.0059	-0.0011	0.0012	0.0011***
Sex(M)	-0.00139	-0.0031	0.0003	0.0009	0.0556
b)					
Intercept (Juvenile)	0.0331	0.0217	0.0446	0.0056	NA
Stage (Adult)	-0.0012	-0.0038	0.0014	0.0013	0.2107
Stage (Nestling)	0.0023	0.0003	0.0043	0.0010	0.0047***
Sex(M)	-0.0014	-0.0031	0.0003	0.0009	0.0568

Note: Parameter estimates, and p-values are from a permutation test and confidence interval and standard error from basic linear mixed effect models, so CI and SE are not reliable.

3.3 Change in inbreeding level within adults

Linear mixed-effect models through permutation testing both with and without a quadratic effect of age were fitted for the full adult data set including Aldra, where the former model

showed a significant decrease in the inbreeding coefficient with age. Similarly, the model including a quadratic effect of age showed there was a general trend for inbreeding to decrease with age, but this trend was not strictly linear as a significant quadratic term indicated that the decrease in inbreeding levels off and slightly reverses at higher ages (see Appendix 4 and Appendix 5). However, upon removing Aldra, the quadratic relationship was not significant anymore. Thus, new data without Aldra was used to fit the linear mixed effect model through permutation with the age of the adult (covariate), sex (factor), and interaction of age and sex as fixed effects, with island and cohort as random factors with random intercepts, the results of which are shown in Table 4

The results showed that at young adult age, females had a higher mean inbreeding coefficient compared to males. The mean inbreeding coefficient significantly decreased with an increase in age by the rate of 0.0034 and 0.0014 per year for females and males respectively. This indicated the relationship between inbreeding and age was stronger for females, so before reaching 2 years old, females had a low inbreeding coefficient compared to males. The results are presented in Figure 4.

Table 4 Parameter estimates of the best model for analyzing the change in inbreeding level with age within adult house sparrows (after removing Aldra).

Parameter	Estimate	Confidence interval		Std. Error	Pr(>|t|)
		2.50%	95%		
Intercept	0.02781	0.02238	0.03306	0.00270	NA
Age	-0.00343	-0.00510	-0.00131	0.00096	0.0000***
Sex(M)	-0.00263	-0.00740	0.00233	0.00248	0.0193***
Age: Sex(M)	0.00175	-0.00078	0.00418	0.00127	0.0017***

Note: Parameter estimates, and p-values are from a permutation test and confidence interval and standard error from basic linear mixed effect models, so CI and SE are not reliable.

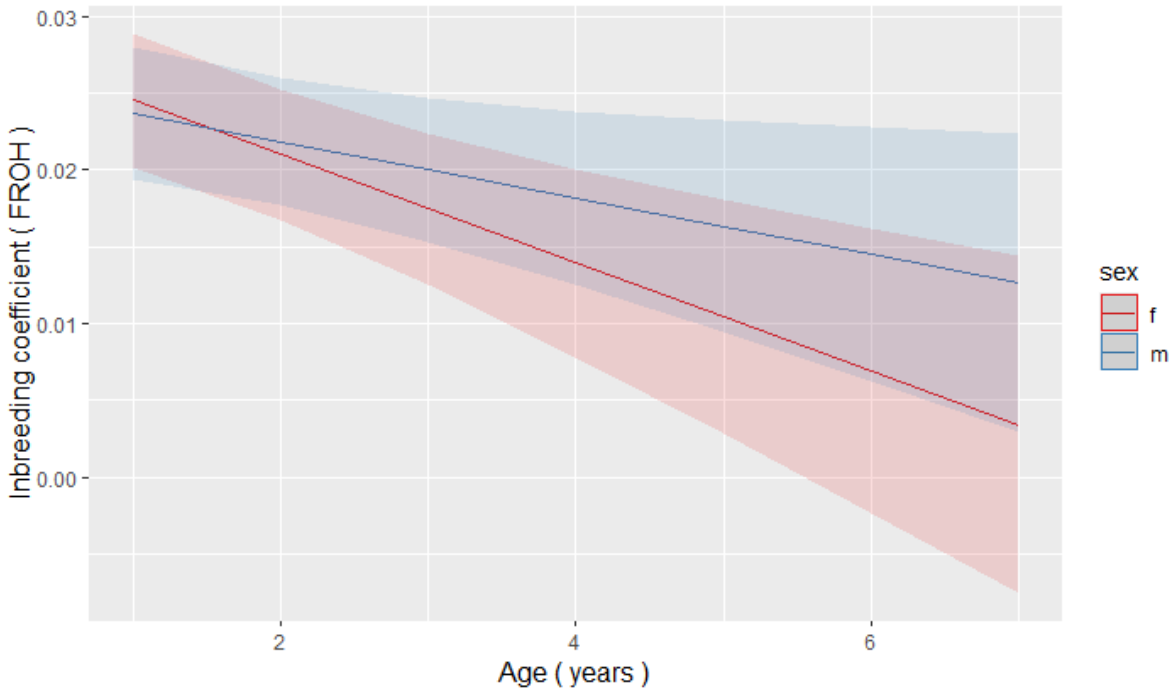


Figure 4 : Change in inbreeding level with age in adult female (in red) and male (in blue) house sparrows.

Note: Confidence intervals are unreliable as they are based on a linear model and not permutation tests.

4. Discussion

In this study, data from 6215 unique house sparrow individuals distributed across the various stages i.e. nestling, juvenile, and adult were used to examine changes in mean inbreeding across life stages and with age within nestlings and adults. Overall, it was found that inbreeding levels decreased both across life stages and with an increase in age for male nestlings and adults of both sexes, consistent with the selective disappearance of inbred individuals that might be from reduced survival due to inbreeding depression.

The results from stage analysis showed that the inbreeding coefficient was higher at the nestling stage than at the juvenile and adult stages. The average inbreeding coefficient significantly decreased from the nestling to the juvenile and adult stages, however, the decrease in inbreeding level was not significant from juvenile to adult. Thus, this indicates inbreeding depression in survival is stronger in the early stage compared to later stages as inbreeding depression is correlated with the inbreeding level (Charlesworth & Charlesworth, 1999; Vega-Trejo et al., 2022). Thus, many inbred individuals might have been removed through high mortality during early life stages as reflected by a decrease in mean inbreeding coefficient from nestling to juvenile and adult stages.

There could be several reasons for inbreeding depression in the survival from nestling to juvenile stage. One reason could be lower body mass caused by inbreeding in nestlings because higher nestling body mass was found to increase the survival (Hajduk et al., 2018) and fledging success of house sparrow (Cleasby et al., 2010; Ringsby et al., 2002). Thus, decreased body mass or inability to increase body mass or maintain growth rate due to reduced competitiveness of highly inbred siblings for parental care or food (De Boer et al., 2016) can contribute to mortality or selective disappearance.

The result from our study is similar to some of the other studies. For example, a study on the bird population of Hihi, *Notiomystis cincta,* found that inbreeding depressed the survival of nestlings (Brekke et al., 2010). A similar trend was found when inbred and non-inbred zebra finch, *Taeniopygia guttata* were compared for survival throughout the life stages (Hemmings

et al., 2012). The survival of hatchlings up to fledging decreased in inbred compared to non-inbred birds, even though it was non-significant. Offspring survival was affected by inbreeding also in other bird species (Brown & Brown, 1998; Daniels & Walters, 2000; Kruuk et al., 2002). A study in Japanese quail bird, *Coturnix coturnix japonica* (Sittmann et al., 1966) found that inbreeding depression at survival was nearly double in early age i.e. zero to five weeks old, compared to later i.e. at five to 16 weeks old.

However, a non-significant decrease in inbreeding levels from juvenile to adult stages means a lack of sufficient disappearance of highly inbred birds to be evident statistically. This might be because inbreeding depression in survival might be comparatively low in later stages as most highly inbred individuals had already died during the nestling stage and from nestling to juvenile stages and may be inbred individuals left in juveniles do not suffer sufficiently from their current level of inbreeding so that further inbreeding-related mortality can be detected statistically. The result is similar to a study from zebra finch, which found no mortality in fledglings to sexual maturity in both inbred and non-inbred populations (Hemmings et al., 2012). A study on the same metapopulation as my study also did not capture any general significant effect of inbreeding level on the recruitment of fledglings unless, the island, hatch date, and sex were considered (Jensen et al., 2007). The study on house sparrows from Aldra also showed a negligible tendency of inbreeding to decrease the recruitment of fledglings (Billing et al., 2012). Also, another study in song sparrows found a similar inbreeding effect in survival of juvenile and adult stages (Keller, 1998). But a study in a reintroduced population of North Island robins, *Petroica longipes* from New Zealand captured the inbreeding depression decreasing the probability of juvenile survival and offspring recruitment into the breeding population (Jamieson et al., 2007).

Thus, inbreeding depression in survival appeared to be at its maximum in the early stage. However, my study did not capture the earliest stages like hatching success or embryo survival. My study lacked data on eggs and early nestlings younger than 4 days old, so most of the effect of inbreeding might not be captured in these analyses as studies in Japanese quail birds (Sittmann et al., 1966) showed most inbreeding depression in embryo development resulting in hatching failure than later stages. So future studies could focus on those initial life stages.

Similarly, another analysis within nestlings was done to see how inbreeding levels change with age. Initially, male nestlings were found to have higher mean inbreeding levels than females. However, the inbreeding coefficient significantly decreased with age in males but there was no significant decrease in females. This might suggest male-biased inbreeding depression or the selective disappearance of highly inbred male nestlings.

This sex-specific difference is similar to the finding from the study of a New Zealand bird, the Hihi, (Brekke et al., 2010), which found male-biased inbreeding depression in the survival of nestlings. It was found that dead male nestlings had higher inbreeding coefficients than dead female nestlings indicating males were more strongly inbred than females at initial days. Also, survived males had lower inbreeding coefficients than survived female nestlings, indicating that inbreeding depression was more pronounced in male nestlings.

There might be several reasons for differential inbreeding depression between sexes, one of them might be sexual size dimorphism (SSD). Male-biased inbreeding depression in nestling might come from SSD as SSD is predicted to cause differences in death rates between sexes (Daniel E.L. et al., 1992). Because of sexual size dimorphism, male nestlings need to grow and acquire high resources rapidly (Brekke et al., 2010) as male nestlings were found to be heavier than their female siblings (Westneat et al., 2002). Also, different endocrine profiles are predicted to create fundamental developmental differences between sexes and differential immune system responses (Benito & González-Solís, 2007). Thus, males may have to face relatively more developmental stress and highly inbred male may not be able to cope as well with stress, which could lead to sex specific selective disappearance of highly inbred males (Brekke et al., 2010), i.e. that inbred males tend to have lower survival.

In contrast, inbreeding levels did not significantly decrease with age in female nestlings, indicating insufficient inbreeding depression in survival. It might be because of less developmental stress for females than males during the age period i.e. 4 to 14 days old.

Moreover, I fitted the model including the quadratic effect of nestling age with an expectation of a curvilinear relation between nestling age and inbreeding coefficient, but the result was unexpected. It showed the inbreeding level increased with age to ca. 7 days of age and after that decreased with age. The biological reason for the unexpected increase in inbreeding with

age initially is unknown and could perhaps be the result of biased sampling. Table 1 shows a disproportionally large number of samples from 4 to 7-day-old nestlings from islands Gjerøy and Hestmannøy, which both have lower than average mean inbreeding levels (Table 5). Thus, this might drive the observed pattern of low average inbreeding level in ages up to 7 days old. So, it could be related to the data structure with an unequal sampling of early and late ages across the different islands and years, even though including island and year as random factors in the models should theoretically account for such effects.

Table 5. Mean inbreeding level in house sparrows based on records in all stages (nestlings, juveniles and adults) on different islands in the study system.

Islands	Mean F_{ROH}
Nesøy	0.04906
Myken	0.04091
Træna	0.01567
Selvær	0.02245
Gjerøy	0.03066
Hestmannøy	0.0153
Indre Kvarøy	0.03196
Lovund	0.01836
Sleneset	0.05217
Aldra	0.08127

Mean F_{ROH} for all islands	0.35782

Similarly, another analysis was done on adults to see if there is any effect of age on inbreeding levels. It was found that inbreeding level decreased with an increase in age in adults. This might reflect mortality of highly inbred adults in the populations over time which, if no new inbred individuals were recruiting to the population, would decrease the average inbreeding level in the adult population. The results suggest there is inbreeding depression in adult survival (De Boer et al., 2018) thus, inbreeding is shortening the average life span of house sparrows in the study system.

There might be various reasons why inbreeding decreases adult survival. Inbreeding showed a tendency to reduce individual body weight in the house sparrow (Niskanen et al., 2020) and low body weight could cause birds to have difficulty coping with harsh and cold weather during winter as energy expenditure is high and periods during the day with sufficient daylight to forage are short, which might affect the survival of high inbreds. As in a population of cliff sallow, *Petrochelidon pyrrhonota*, larger body sizes were favored in the cold spell of 1996 (Brown & Brown, 1998). Highly inbred birds might not be able to cope with the harsh winter as inbreeding depression increases with environmental stress (Armbruster & Reed, 2005; Fox & Reed, 2011) as there might be inbreeding and environmental interaction (Kristensen et al., 2006). Both inbreeding and environmental stress, e.g. extreme cold, independently alter gene expression. When these stresses co-occur, their interaction may further cause instability of gene expression interrupting the stress response mechanism and the metabolic function (Kristensen et al., 2006) causing mortality. Also, stress may increase the expression of deleterious recessive alleles that decrease individual fitness. A study on *Drosophila melanogaster* (Miller, 1994) suggests high inbreeding depression in stressful environments. Another study on Drosophila shows high inbreeding depression in egg-adult viability at extremely low temperatures compared to intermediate temperatures in semi-natural conditions, and more inbreeding depression in both high and low temperatures at laboratory conditions (Kristensen et al., 2008) indicating an interaction between inbreeding and the environment.

This means the intensity of inbreeding depression may vary in different environments. Another reason might be because of harsh winters there may be food scarcity, and highly inbred adults might not be able to compete or search for food compared to low-inbred or non-inbred individuals and may die. Also, senescence and inbreeding collectively might contribute to shorter life spans of inbred adults.

The decreased adult survival in my study is similar to another study on cactus finches, *Geospiza scandens*. In finches, it was found that inbred adults with an inbreeding coefficient of 0.25 have a 45% reduction in annual survival (Keller et al., 2002). Similarly, in a study of the same house sparrow metapopulation, it has been found that inbreeding decreases adult survival (Niskanen et al., 2020). In contrast, another study of house sparrows from the island Aldra did not find evidence of the effect of inbreeding on adult survival and lifespan (Billing et al., 2012).

In addition, there was also an interaction between age and sex which means the decrease in inbreeding level with age also depends on the sexes. It was seen that initially, males had a lower mean inbreeding coefficient, this might be because, in the nestling stage, the inbreeding level had decreased at a higher rate in males compared to females removing highly inbred male nestlings and at the juvenile stage also male have low level of inbreeding. However, F_{ROH} decreased faster with age in females than in males, indicating stronger inbreeding depression in the survival of females. This might be a result of more inbred females surviving from earlier stages, as the result from nestling analysis showed there was no decrease in inbreeding level for female nestlings. So, after reaching adulthood, high inbreeding levels might interact with other stress causing females to die. This might be because egg production and laying are energy-demanding (Nilsson & Råberg, 2001) so, females might have higher mortality due to the costs of reproduction (Romano et al., 2022). Such energy-demanding egg production can lead to the depletion of protein and lipids from tissue (Houston et al., 1995; Williams & Martyniuk, 2000) or reduction of body reserves and flight muscles. This increases the vulnerability to parasite infection and impairs flight performance leading to high predation risk. These effects can be intense in highly inbred females causing them to die more rapidly and thus decreasing the mean inbreeding level of females in the population.

However, the result from this study contrasts with studies that showed a greater impact of inbreeding on longevity for adult males compared to females (Keller et al., 2008; Trask et al., 2021). Also, a study on the same house sparrow metapopulation showed the annual survival rate between sexes was not different but varied between islands and years (Ringsby et al., 1999). So, there might be differences between the sexes in this study period 2007- 2014.

Inbreeding has been a huge problem for the viability of small and endangered populations or species (Charlesworth & Charlesworth, 1999). So, these populations' conservation also needs to focus on the conservation genetics perspective. Quantifying inbreeding depression can be quite challenging as measuring fitness and its components are both resource and time-consuming. So, monitoring the inbreeding level in the various life stages through sampling of individuals at different life-history stages and ages within those stages by estimating the genomic inbreeding coefficient can give an idea about the general fitness of the population as fitness is correlated to the inbreeding level. Regular monitoring of the inbreeding level also gives an idea about the effectiveness of the management and conservation strategies. Thus, conservation and management strategies can be modified accordingly, to reduce the inbreeding level and improve the population viability.

Similarly, studying inbreeding across life stages can be quite challenging in most of the populations because sufficient sample sizes may be difficult to obtain. However, in the house sparrow metapopulation study system in northern Norway, a large data set has been collected over the years. So, the findings from this study can be implicated in other animal species that do not have sufficient sample sizes for reliable analyses. In case, any species or population is critically endangered and about to become extinct, then management activities should focus on maintaining an optimum environment to reduce mortality in early life stages. In addition, there should be innovative approaches to increase gene flow through habitat connectivity or translocating individuals from different populations so genetically unrelated mates can be available which reduces inbreeding in later generations.

As inbreeding depression reduces adult survival, it can lower the number of breeding pairs in the population. Besides, several studies found that inbreeding decreases reproductive success (Billing et al., 2012; Niskanen et al., 2020). These things can have a cumulative effect in

reducing population size. Thus, we must focus on reducing the inbreeding level through genetic rescue to sustain a healthy population. It can be done by a translocation program or by improving the connectivity of habitats for gene flow in the first place and can support the species through habitat management.

4.1 Limitations

This study has some limitations. It uses a permutation method to fit the model to test for changes in inbreeding levels. Because of this, the confidence intervals of parameter estimates presented are from linear mixed effect models and do not represent the accurate value thus, cannot be relied upon. Also, this study uses data from nestling, juvenile, and adult stages. Within the nestling stage, there is data only from the earliest 4 days. Thus, valuable information from earlier life-history stages like the egg, hatching, or young nestling (i.e. up to 3 days old) is absent. The selective disappearance of highly inbred individuals might have occurred most in that period; thus, this study could not capture it. Moreover, the data collected from 10 different islands and years/cohorts in various stages and ages were not of similar sizes, which might have affected the result from the model even though islands and years are specified as random factors. Future studies could be done considering these things.

4.2 Conclusion

In conclusion, there is a decrease in inbreeding levels in the population from the early stage to the later stage or with an increase in age that indicates the selective disappearance of inbred individuals because of inbreeding depression in survival. Inbreeding depression in survival could result from the expression of deleterious recessive alleles due to increased homozygosity in inbred individuals (Charlesworth & Willis, 2009). This inbreeding depression appeared to be most severe in the early stages suggesting intensive management activities should focus on early stages in conservation projects, especially in endangered or critically endangered species. Moreover, the inbreeding depression also varied based on sex and stages.

5. References

Allendorf, F. W., Hohenlohe, P. A., & Luikart, G. (2010). Genomics and the future of conservation genetics. *Nature Reviews Genetics*, *11*(10), 697–709. https://doi.org/10.1038/nrg2844

Anderson, T. R. (2006). Biology of the ubiquitous house sparrow: From genes to populations. Oxford University Press.

Armbruster, P., & Reed, D. H. (2005). Inbreeding depression in benign and stressful environments. *Heredity*, *95*(3), 235–242. https://doi.org/10.1038/sj.hdy.6800721

Baalsrud, H. T., Sæther, B., Hagen, I. J., Myhre, A. M., Ringsby, T. H., Pärn, H., & Jensen, H. (2014). Effects of population characteristics and structure on estimates of effective population size in a house sparrow metapopulation. *Molecular Ecology*, *23*(11), 2653–2668. https://doi.org/10.1111/mec.12770

Ballou, J. D. (1983). Calculating inbreeding from pedigree.

Balloux, F., Amos, W., & Coulson, T. (2004). Does heterozygosity estimate inbreeding in real populations? *Molecular Ecology*, *13*(10), 3021–3031. https://doi.org/10.1111/j.1365-294X.2004.02318.x

Billing, A. M., Lee, A. M., Skjelseth, S., Borg, Å. A., Hale, M. C., Slate, J., Pärn, H., Ringsby, T. H., Sæther, B., & Jensen, H. (2012). Evidence of inbreeding depression but not

inbreeding avoidance in a natural house sparrow population. *Molecular Ecology*, *21*(6), 1487–1499. https://doi.org/10.1111/j.1365-294X.2012.05490.x

Brekke, P., Bennett, P. M., Wang, J., Pettorelli, N., & Ewen, J. G. (2010). Sensitive males: Inbreeding depression in an endangered bird. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1700), 3677–3684. https://doi.org/10.1098/rspb.2010.1144

Brooks, M., Bolker, B., Kristensen, K., & Maechler, M. (2024). Generalized Linear Mixed Models using Template Model Builder. https://cran.r-project.org/package=glmmTMB

Brooks, T. M., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A. B., Rylands, A. B., Konstant, W. R., Flick, P., Pilgrim, J., Oldfield, S., Magin, G., & Hilton-Taylor, C. (2002). Habitat Loss and Extinction in the Hotspots of Biodiversity. *Conservation Biology*, *16*(4), 909–923. https://doi.org/10.1046/j.1523-1739.2002.00530.x

Brown, C. R., & Brown, M. B. (1998). Intense natural selection on body size and wing and tail asymmetry in cliff swallows during severe weather. *Evolution*, *52*(5), 1461–1475. https://doi.org/10.1111/j.1558-5646.1998.tb02027.x

Brown, J. L., & Brown, E. R. (1998). Are inbred offspring less fit? Survival in a natural population of Mexican jays. *Behavioral Ecology*, *9*(1), 60–63. https://doi.org/10.1093/beheco/9.1.60

Caughley, G. (1994). Directions in Conservation Biology. *The Journal of Animal Ecology*, *63*(2), 215. https://doi.org/10.2307/5542

Chang, C. C., Chow, C. C., Tellier, L. C., Vattikuti, S., Purcell, S. M., & Lee, J. J. (2015). Second-generation PLINK: Rising to the challenge of larger and richer datasets. *GigaScience*, *4*(1), 7. https://doi.org/10.1186/s13742-015-0047-8

Charlesworth, B. (2018). Mutational load, inbreeding depression and heterosis in subdivided populations. *Molecular Ecology*, *27*(24), 4991–5003. https://doi.org/10.1111/mec.14933

Charlesworth, B., & Charlesworth, D. (1999). The genetic basis of inbreeding depression. *Genetical Research*, *74*(3), 329–340. https://doi.org/10.1017/S0016672399004152

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

Cleasby, I. R., Nakagawa, S., Gillespie, D. O. S., & Burke, T. (2010). The influence of sex and body size on nestling survival and recruitment in the house sparrow: juvenile survival in the house sparrow. *Biological Journal of the Linnean Society*, *101*(3), 680–688. https://doi.org/10.1111/j.1095-8312.2010.01515.x

Cordero, P. J., Wetton, J. H., & Parkin, D. T. (1999). Extra-Pair Paternity and Male Badge Size in the House Sparrow. *Journal of Avian Biology*, *30*(1), 97. https://doi.org/10.2307/3677248

Daniel E.L., P., Martin, T., & Robert, Montgomerie. (1992). Mortality costs of sexual dimorphism in birds. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *250*(1328), 143–150. https://doi.org/10.1098/rspb.1992.0142

Daniels, S. J., & Walters, J. R. (2000). Inbreeding Depression and its Effects on Natal Dispersal in Red-Cockaded Woodpeckers. *The Condor*, *102*(3), 482–491. https://doi.org/10.1093/condor/102.3.482

De Boer, R. A., Eens, M., & Müller, W. (2016). A loss of heterozygosity, a loss in competition? The effects of inbreeding, pre- and postnatal conditions on nestling development. *Ecology and Evolution*, *6*(21), 7921–7930. https://doi.org/10.1002/ece3.2496

De Boer, R. A., Eens, M., & Müller, W. (2018). Sex-specific effects of inbreeding on reproductive senescence. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1879), 20180231. https://doi.org/10.1098/rspb.2018.0231

Demontis, D., Pertoldi, C., Loeschcke, V., Mikkelsen, K., Axelsson, T., & Kristensen, T. N. (2009). Efficiency of selection, as measured by single nucleotide polymorphism variation, is dependent on inbreeding rate in *Drosophila melanogaster*. *Molecular Ecology*, *18*(22), 4551–4563. https://doi.org/10.1111/j.1365-294X.2009.04366.x

DeRose, M. A., & Roff, D. A. (1999). A comparison of inbreeding depression in life-history and morphological traits in animals. *Evolution*, *53*(4), 1288–1292. https://doi.org/10.1111/j.1558-5646.1999.tb04541.x

Dobzhansky, T. (1950). Genetics of natural populations. Xix. Origin of heterosis through natural selection in populations of drosophila pseudoobscura. *Genetics*, *35*(3), 288–302. https://doi.org/10.1093/genetics/35.3.288

Fox, C. W., & Reed, D. H. (2011). Inbreeding depression increases with environmental stress: an experimental study and meta-analysis: inbreeding load increases with stress. *Evolution*, *65*(1), 246–258. https://doi.org/10.1111/j.1558-5646.2010.01108.x

Frankham, R. (2022). Evaluation of proposed genetic goals and targets for the Convention on Biological Diversity. *Conservation Genetics*, *23*(5), 865–870. https://doi.org/10.1007/s10592-022-01459-1

Frankham, R., David A., . Briscoe, & onathan D., B. (2002). *Introduction to conservation genetics*. Cambridge University Press.

Gibson, J., Morton, N. E., & Collins, A. (2006). Extended tracts of homozygosity in outbred human populations. *Human Molecular Genetics*, *15*(5), 789–795. https://doi.org/10.1093/hmg/ddi493

Girndt, A., Chng, C. W. T., Burke, T., & Schroeder, J. (2018). Male age is associated with extra-pair paternity, but not with extra-pair mating behaviour. *Scientific Reports*, *8*(1), 8378. https://doi.org/10.1038/s41598-018-26649-1

Grueber, C. E., Laws, R. J., Nakagawa, S., & Jamieson, I. G. (2010). Inbreeding Depression Accumulation across Life-History Stages of the Endangered Takahe. *Conservation Biology*, *24*(6), 1617–1625. https://doi.org/10.1111/j.1523-1739.2010.01549.x

Hajduk, G. K., Cockburn, A., Margraf, N., Osmond, H. L., Walling, C. A., & Kruuk, L. E. B. (2018). Inbreeding, inbreeding depression, and infidelity in a cooperatively breeding bird*: inbreeding and infidelity. *Evolution*, *72*(7), 1500–1514. https://doi.org/10.1111/evo.13496

Hanski, I. (2005). Landscape fragmentation, biodiversity loss and the societal response: The longterm consequences of our use of natural resources may be surprising and unpleasant. *EMBO Reports*, *6*(5), 388–392. https://doi.org/10.1038/sj.embor.7400398

Harrisson, K. A., Magrath, M. J. L., Yen, J. D. L., Pavlova, A., Murray, N., Quin, B., Menkhorst, P., Miller, K. A., Cartwright, K., & Sunnucks, P. (2019). Lifetime Fitness Costs of Inbreeding and Being Inbred in a Critically Endangered Bird. *Current Biology*, *29*(16), 2711-2717.e4. https://doi.org/10.1016/j.cub.2019.06.064

Hartig, F. (2022). Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. https://cran.r-project.org/package=DHARMa

Hedrick, P. W., & Garcia-Dorado, A. (2016). Understanding Inbreeding Depression, Purging, and Genetic Rescue. *Trends in Ecology & Evolution*, *31*(12), 940–952. https://doi.org/10.1016/j.tree.2016.09.005

Hedrick, P. W., & Kalinowski, S. T. (2000). Inbreeding Depression in Conservation Biology. *Annual Review of Ecology and Systematics*, *31*(1), 139–162. https://doi.org/10.1146/annurev.ecolsys.31.1.139

Hemmings, N. L., Slate, J., & Birkhead, T. R. (2012). Inbreeding causes early death in a passerine bird. *Nature Communications*, *3*(1), 863. https://doi.org/10.1038/ncomms1870

Houston, D. C., Donnan, D., & Jones, P. J. (1995). The source of the nutrients required for egg production in zebra finches *Poephila guttata*. *Journal of Zoology*, *235*(3), 469–483. https://doi.org/10.1111/j.1469-7998.1995.tb01763.x

Huisman, J., Kruuk, L. E. B., Ellis, P. A., Clutton-Brock, T., & Pemberton, J. M. (2016). Inbreeding depression across the lifespan in a wild mammal population. *Proceedings of the National Academy of Sciences*, *113*(13), 3585–3590. https://doi.org/10.1073/pnas.1518046113

Husby, A., Sæther, B., Jensen, H., & Ringsby, T. H. (2006). Causes and consequences of adaptive seasonal sex ratio variation in house sparrows. *Journal of Animal Ecology*, *75*(5), 1128–1139. https://doi.org/10.1111/j.1365-2656.2006.01132.x

Jamieson, I. G., Tracy, L. N., Fletcher, D., & Armstrong, D. P. (2007). Moderate inbreeding depression in a reintroduced population of North Island robins. *Animal Conservation*, *10*(1), 95–102. https://doi.org/10.1111/j.1469-1795.2006.00078.x

Jensen, H., Bremset, E. M., Ringsby, T. H., & Sæther, B. (2007). Multilocus heterozygosity and inbreeding depression in an insular house sparrow metapopulation. *Molecular Ecology*, *16*(19), 4066–4078. https://doi.org/10.1111/j.1365-294X.2007.03452.x

Jump, A. S., Marchant, R., & Peñuelas, J. (2009). Environmental change and the option value of genetic diversity. *Trends in Plant Science*, *14*(1), 51–58. https://doi.org/10.1016/j.tplants.2008.10.002

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

Keller, L. (2002). Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, *17*(5), 230–241. https://doi.org/10.1016/S0169-5347(02)02489-8

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

Keller, L. F., Grant, P. R., Grant, B. R., & Petren, K. (2002). Environmental conditions affect the magnitude of inbreeding depression in survival of darwin's finches. *Evolution*, *56*(6), 1229–1239. https://doi.org/10.1111/j.0014-3820.2002.tb01434.x

Keller, L. F., Reid, J. M., & Arcese, P. (2008). Testing evolutionary models of senescence in a natural population: Age and inbreeding effects on fitness components in song

sparrows. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1635), 597–604. https://doi.org/10.1098/rspb.2007.0961

Keller, M. C., Visscher, P. M., & Goddard, M. E. (2011). Quantification of Inbreeding Due to Distant Ancestors and Its Detection Using Dense Single Nucleotide Polymorphism Data. *Genetics*, *189*(1), 237–249. https://doi.org/10.1534/genetics.111.130922

Kristensen, T. N., Barker, J. S. F., Pedersen, K. S., & Loeschcke, V. (2008). Extreme temperatures increase the deleterious consequences of inbreeding under laboratory and semi-natural conditions. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1646), 2055–2061. https://doi.org/10.1098/rspb.2008.0426

Kristensen, T. N., Sørensen, P., Pedersen, K. S., Kruhøffer, M., & Loeschcke, V. (2006). Inbreeding by Environmental Interactions Affect Gene Expression in *Drosophila melanogaster*. *Genetics*, *173*(3), 1329–1336. https://doi.org/10.1534/genetics.105.054486

Kruuk, L. E. B., Sheldon, B. C., & Merilä, J. (2002). Severe inbreeding depression in collared flycatchers (*Ficedula albicollis*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1500), 1581–1589. https://doi.org/10.1098/rspb.2002.2049

Lande, R., & Schemske, D. W. (1985). The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution*, *39*(1), 24–40. https://doi.org/10.1111/j.1558-5646.1985.tb04077.x

Leimu, R., Mutikainen, P., Koricheva, J., & Fischer, M. (2006). How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology*, *94*(5), 942–952. https://doi.org/10.1111/j.1365-2745.2006.01150.x

Manel, S., & Holderegger, R. (2013). Ten years of landscape genetics. *Trends in Ecology & Evolution*, *28*(10), 614–621. https://doi.org/10.1016/j.tree.2013.05.012

Mastrangelo, S., Tolone, M., Di Gerlando, R., Fontanesi, L., Sardina, M. T., & Portolano, B. (2016). Genomic inbreeding estimation in small populations: Evaluation of runs of homozygosity in three local dairy cattle breeds. *Animal*, *10*(5), 746–754. https://doi.org/10.1017/S1751731115002943

McQuillan, R., Leutenegger, A.-L., Abdel-Rahman, R., Franklin, C. S., Pericic, M., Barac-Lauc, L., Smolej-Narancic, N., Janicijevic, B., Polasek, O., Tenesa, A., MacLeod, A. K., Farrington, S. M., Rudan, P., Hayward, C., Vitart, V., Rudan, I., Wild, S. H., Dunlop, M. G., Wright, A. F., … Wilson, J. F. (2008). Runs of Homozygosity in European Populations. *The American Journal of Human Genetics*, *83*(3), 359–372. https://doi.org/10.1016/j.ajhg.2008.08.007

Miller, P. S. (1994). Is inbreeding depression more severe in a stressful environment? *Zoo Biology*, *13*(3), 195–208. https://doi.org/10.1002/zoo.1430130302

Muller, M.-L. D., Dutang, C., & Siberchicot, A. (2023). *Help to Fit of a Parametric Distribution to Non-Censored or Censored Data*. https://cran.r-project.org/package=fitdistrplus

Mullu, D. (2016). A Review on the Effect of Habitat Fragmentation on Ecosystem.

Nelson, W. A., & Crone, E. E. (1999). Genetics and Analysis of Quantitative Traits. Michael Lynch , Bruce Walsh. *The Quarterly Review of Biology*, *74*(2), 225–225. https://doi.org/10.1086/393101

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

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

Ranke, P. S., Araya-Ajoy, Y. G., Ringsby, T. H., Pärn, H., Rønning, B., Jensen, H., Wright, J., & Sæther, B. (2021). Spatial structure and dispersal dynamics in a house sparrow metapopulation. *Journal of Animal Ecology*, *90*(12), 2767–2781. https://doi.org/10.1111/1365-2656.13580

Ringsby, T. H., Saether, B.-E., Altwegg, R., & Solberg, E. J. (1999). Temporal and Spatial Variation in Survival Rates of a House Sparrow, Passer domesticus, Metapopulation. *Oikos*, *85*(3), 419. https://doi.org/10.2307/3546691

Ringsby, T. H., Saether, B.-E., & Solberg, E. J. (1998). Factors Affecting Juvenile Survival in House Sparrow Passer domesticus. *Journal of Avian Biology*, *29*(3), 241. https://doi.org/10.2307/3677106

Ringsby, T. H., Sæther, B.-E., Tufto, J., Jensen, H., & Solberg, E. J. (2002). Asynchronous spatiotemporal demography of a house sparrow metapopulation in a correlated environment. *Ecology*, *83*(2), 561–569. https://doi.org/10.1890/0012-9658(2002)083[0561:ASDOAH]2.0.CO;2

Robinson, S. K., Thompson, F. R., Donovan, T. M., Whitehead, D. R., & Faaborg, J. (1995). Regional Forest Fragmentation and the Nesting Success of Migratory Birds. *Science*, *267*(5206), 1987–1990. https://doi.org/10.1126/science.267.5206.1987

Roff, D. A. (1998). Effects of inbreeding on morphological and life history traits of the sand cricket, Gryllus firmus. *Heredity*, *81*(1), 28–37. https://doi.org/10.1046/j.1365-2540.1998.00363.x

Romano, A., Liker, A., Bazzi, G., Ambrosini, R., Møller, A. P., & Rubolini, D. (2022). Annual egg productivity predicts female-biased mortality in avian species. *Evolution*, *76*(11), 2553–2565. https://doi.org/10.1111/evo.14623

Saatoglu, D., Niskanen, A. K., Kuismin, M., Ranke, P. S., Hagen, I. J., Araya-Ajoy, Y. G., Kvalnes, T., Pärn, H., Rønning, B., Ringsby, T. H., Sæther, B., Husby, A., Sillanpää, M. J., & Jensen, H. (2021). Dispersal in a house sparrow metapopulation: An integrative case study of genetic assignment calibrated with ecological data and pedigree information. *Molecular Ecology*, *30*(19), 4740–4756. https://doi.org/10.1111/mec.16083

Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W., & Hanski, I. (1998). Inbreeding and extinction in a butterfly metapopulation. *Nature*, *392*(6675), 491–494. https://doi.org/10.1038/33136

Schlaepfer, D. R., Braschler, B., Rusterholz, H., & Baur, B. (2018). Genetic effects of anthropogenic habitat fragmentation on remnant animal and plant populations: A meta-analysis. *Ecosphere*, *9*(10), e02488. https://doi.org/10.1002/ecs2.2488

Sittmann, K., Abplanalp, H., & Fraser, R. A. (1966). Inbreeding depression in japanese quail. *Genetics*, *54*(2), 371–379. https://doi.org/10.1093/genetics/54.2.371

Taylor, S. S., Sardell, R. J., Reid, J. M., Bucher, T., Taylor, N. G., Arcese, P., & Keller, L. F. (2010). Inbreeding coefficient and heterozygosity-fitness correlations in unhatched and hatched song sparrow nestmates: inbreeding coefficient and heterozygosity-fitness correlations. *Molecular Ecology*, *19*(20), 4454–4461. https://doi.org/10.1111/j.1365-294X.2010.04824.x

Trask, A. E., Ferrie, G. M., Wang, J., Newland, S., Canessa, S., Moehrenschlager, A., Laut, M., Duenas, L. B., & Ewen, J. G. (2021). Multiple life-stage inbreeding depression impacts demography and extinction risk in an extinct-in-the-wild species. *Scientific Reports*, *11*(1), 682. https://doi.org/10.1038/s41598-020-79979-4

Vaclav, R. (2003). Food supplementation affects extrapair paternity in house sparrows (Passer domesticus). *Behavioral Ecology*, *14*(5), 730–735. https://doi.org/10.1093/beheco/arg059

VanRaden, P. M., Olson, K. M., Wiggans, G. R., Cole, J. B., & Tooker, M. E. (2011). Genomic inbreeding and relationships among Holsteins, Jerseys, and Brown Swiss. *Journal of Dairy Science*, *94*(11), 5673–5682. https://doi.org/10.3168/jds.2011-4500

Voeten, r C. C. (2023). *Permutation Tests for Time Series Data*. https://cran.r-project.org/package=permutes

Westneat, D. F., Stewart, I. R. K., Woeste, E. H., Gipson, J., Abdulkadir, L., & Poston, J. P. (2002). Patterns of Sex Ratio Variation in House Sparrows. *The Condor*, *104*(3), 598–609. https://doi.org/10.1093/condor/104.3.598

Williams, T. D., & Martyniuk, C. J. (2000). Tissue mass dynamics during egg-production in female Zebra Finches *Taeniopygia guttata*: Dietary and hormonal manipulations. *Journal of Avian Biology*, *31*(1), 87–95. https://doi.org/10.1034/j.1600-048X.2000.310112.x

Wright, L. I., Tregenza, T., & Hosken, D. J. (2008). Inbreeding, inbreeding depression and extinction. *Conservation Genetics*, *9*(4), 833–843. https://doi.org/10.1007/s10592-007-9405-0

Wright, S. (1922). Coefficients of Inbreeding and Relationship. *The American Naturalist*, *56*(645), 330–338. https://doi.org/10.1086/279872

WWF. (2022). *Living Planet Report 2022*. https://wwfint.awsassets.panda.org/downloads/embargo_13_10_2022_lpr_2022_full_report_single_page_1.pdf

6. Appendix

Appendix 1 Distribution of sampled data in different stages and islands.

Islands Stages	Nesøy	Myken	Træna	Selvær	Gjerøy	Hest-mannøy	Indre Kvarøy	Lovund	Sleneset	Aldra	Total
Nestling	266	204	711	618	786	949	404	271	433	143	4785
Juvenile	52	104	515	251	516	773	335	167	189	99	3001
Adult	37	41	253	183	342	461	165	110	75	61	1728
Total	355	349	1479	1052	1644	2183	904	548	697	303	9514

Appendix 2 Distribution of data in different stages.

Stages	Sampled no. of individuals (unique within each stage)	No. of unique individuals across whole stages	Duplicated individuals across whole stages
Nestlings	4785	4785	0
Juvenile	3001	1140	1861
Adult	1728	290	1438
Total	9514	6215	3299

Note: sampled no of individuals refers to unique individuals within each stage, unique individuals mean

individuals not repeated in other stages too i.e. unique in whole data.

Appendix 3 Distribution of adult records in different ages and islands.

Islands / Age in yrs.	Nesøy	Myken	Træna	Selvær	Gjerøy	Hest-mannøy	Indre Kvarøy	Lovund	Sleneset	Aldra	Total
1	34	39	234	164	331	433	154	105	68	59	161
2	16	17	81	61	156	193	54	42	26	20	666
3	5	6	23	29	71	100	30	10	9	11	294
4	0	3	12	18	30	39	5	4	6	4	121
5	1	0	4	4	10	10	6	3	0	3	41
6	0	0	3	2	6	2	4	0	1	1	19
7	0	0	0	2	1	0	0	0	1	1	5
Total	56	65	357	280	605	777	253	164	111	99	2767

Appendix 4 Parameter estimates for the linear mixed effect model with quadratic effect of age were also included for analyzing how the inbreeding coefficient changes with age within adults with full data including Aldra.

Parameter	Estimate	Confidence interval		Std. Error	Pr(>|t|)
		2.50%	95%		
Intercept	0.03198	0.0209	0.0431	0.0055	NA
Age	-0.00297	-0.0076	0.0017	0.0024	0.000***
I(Age^2)	0.00027	-0.0005	0.0011	0.0004	0.0177***
Sex(M)	-0.00138	-0.0063	0.0036	0.0025	0.1445
Age: Sex(M)	-0.00001	-0.0025	0.0025	0.0013	1

Note: Parameter estimates, and p-values are from permutation test and confidence interval and standard error from basic linear mixed effect models, so CI and SE are not reliable.

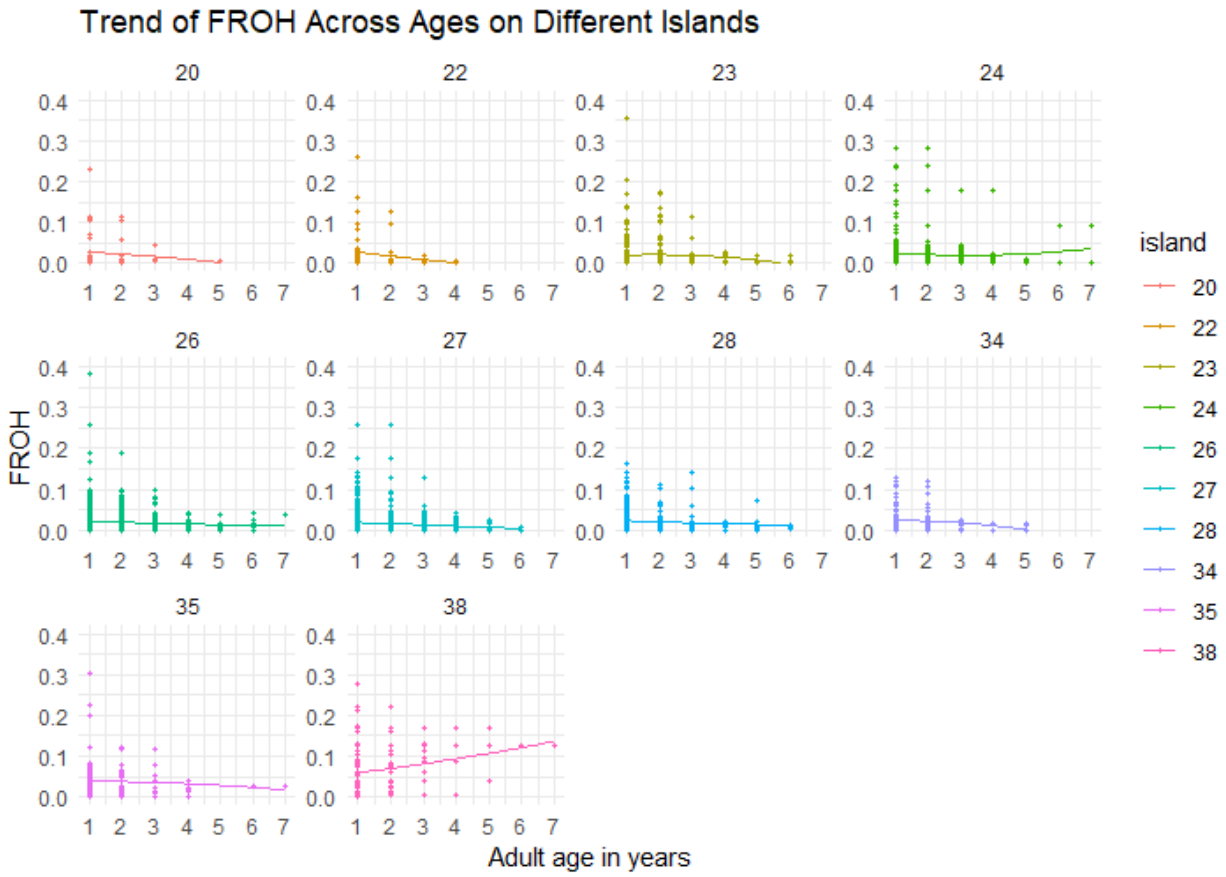
Appendix 5 Parameter estimates of a model without a quadratic term used for analyzing how the inbreeding coefficient changes with age within adult with data from all islands in the study.

Parameter	Estimate	Confidence interval		Std. Error	Pr(>|t|)
		2.50%	95%		
Intercept	0.03072	0.02025	0.04125	0.00519	NA
Age	-0.00159	0.00348	0.00031	0.00097	0.0045***
Sex(M)	-0.00143	0.00639	0.00354	0.00254	0.1411

Age: Sex(M)	0.00001	0.00252	0.00253	0.00129	1

Note: Parameter estimates, and p-values are from permutation test and confidence interval and standard error from basic linear mixed effect models, so CI and SE are not reliable.

Appendix 6 Change in inbreeding level with age of adults in different islands.

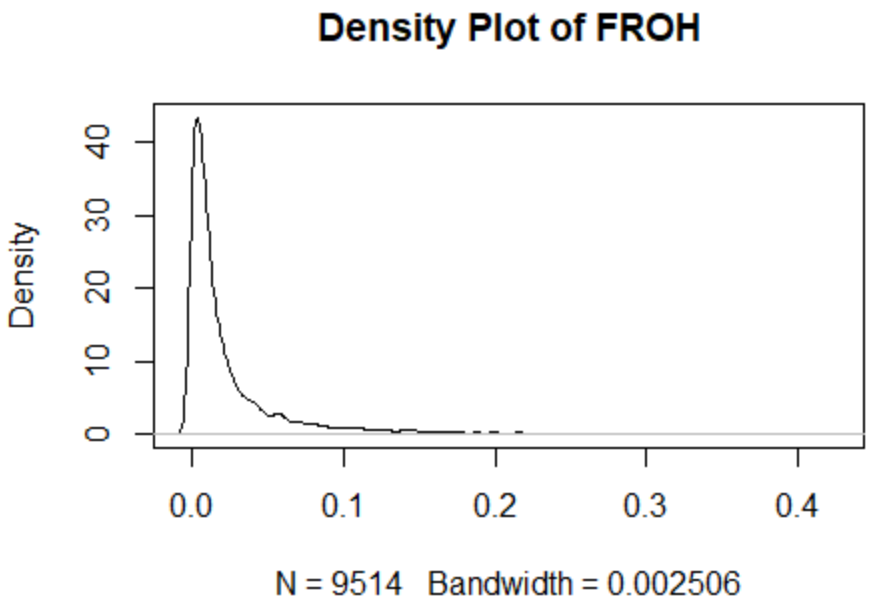


Appendix 7 Parameter estimates of linear mixed effect model with quadratic effect of age included for analyzing how the inbreeding coefficient changes with age within adults with the data excluding Aldra.

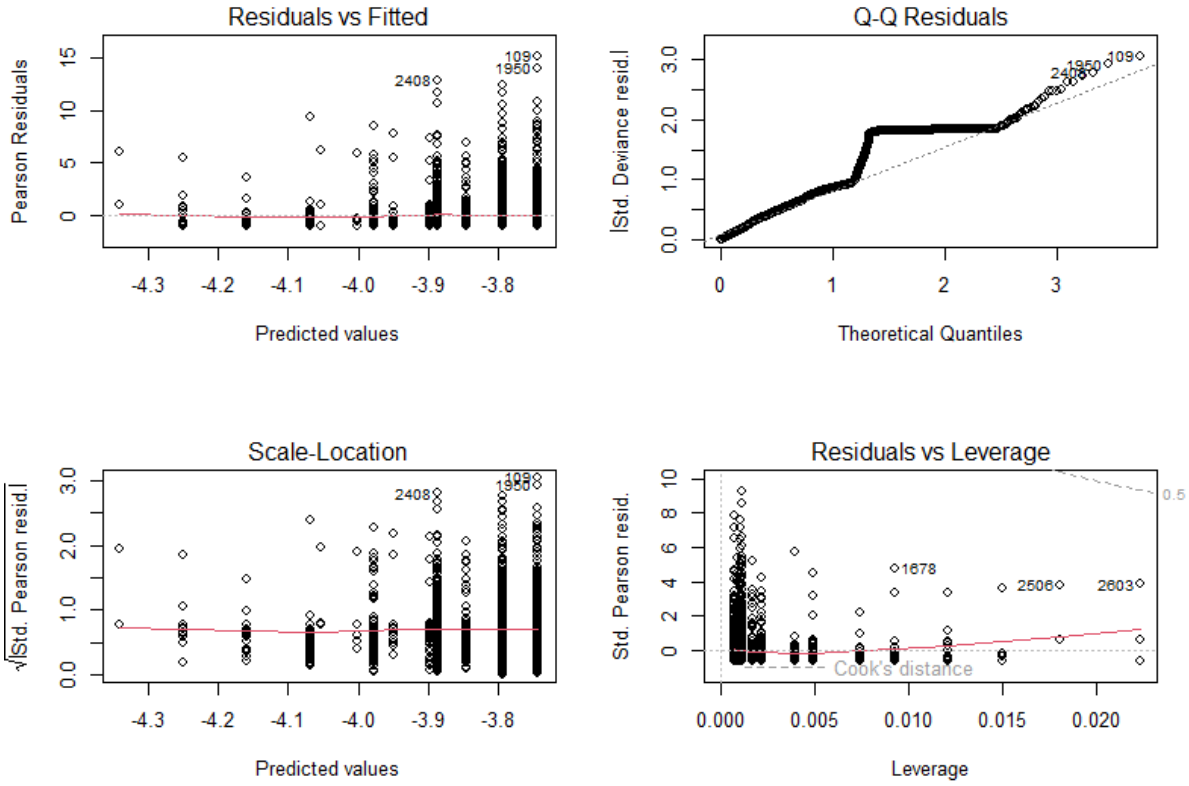
Parameter	Estimate	Confidence interval		Std. Error	Pr(>|t|)
		2.50%	95%		
Intercept	0.02829	0.02139	0.03433	0.00331	NA
Age	-0.00395	0.00798	0.00113	0.00232	0.000***
I(Age^2)	0.00010	0.00077	0.00086	0.00042	0.210
Sex(M)	-0.00259	0.00739	0.00235	0.00249	0.022**
Age: Sex(M)	0.00173	0.00080	0.00418	0.00127	0.001***

Note: Parameter estimate, and p-values are from permutation test and confidence interval and standard error from basic linear mixed effect models, so CI and SE are not reliable.

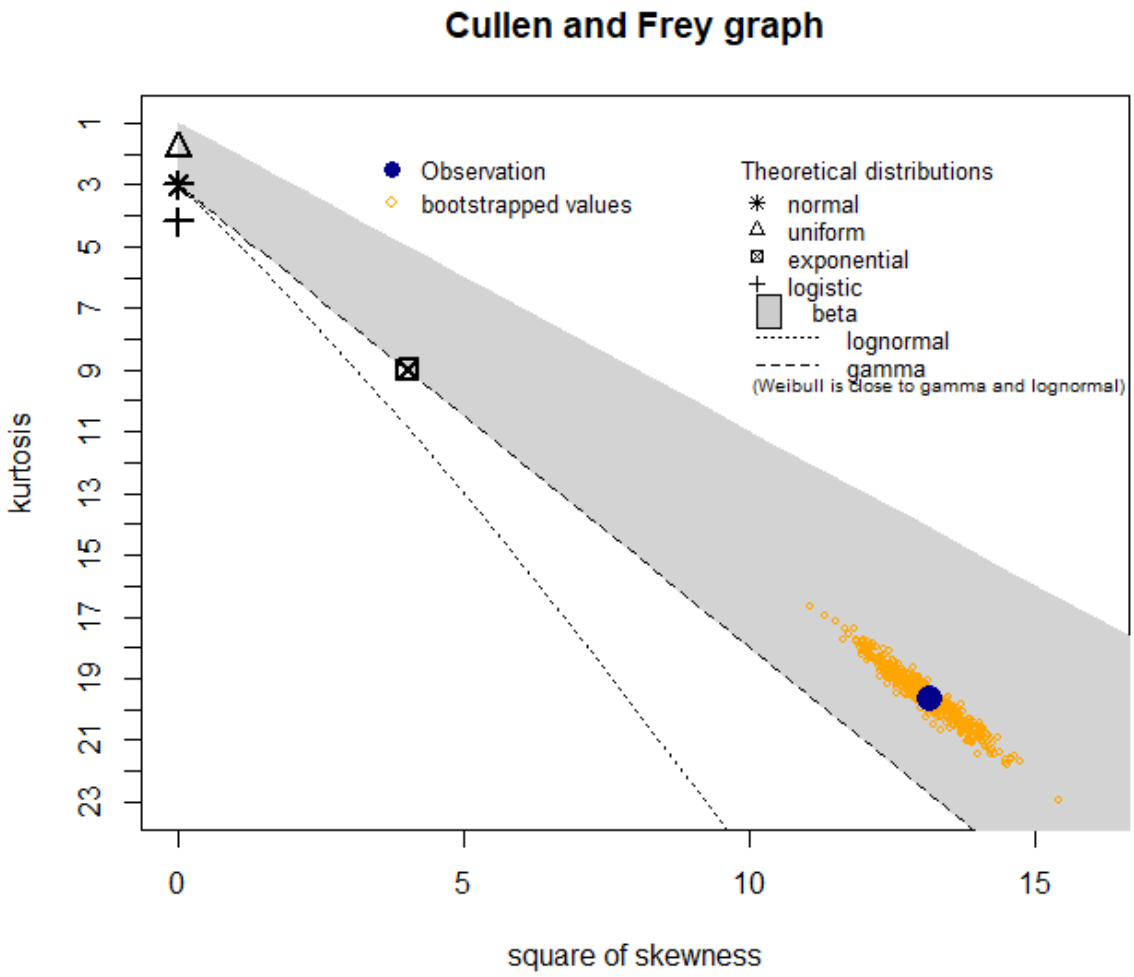
Appendix 8 Density plot of individual inbreeding coefficients.



Appendix 9 Model diagnostic for a generalized linear model.



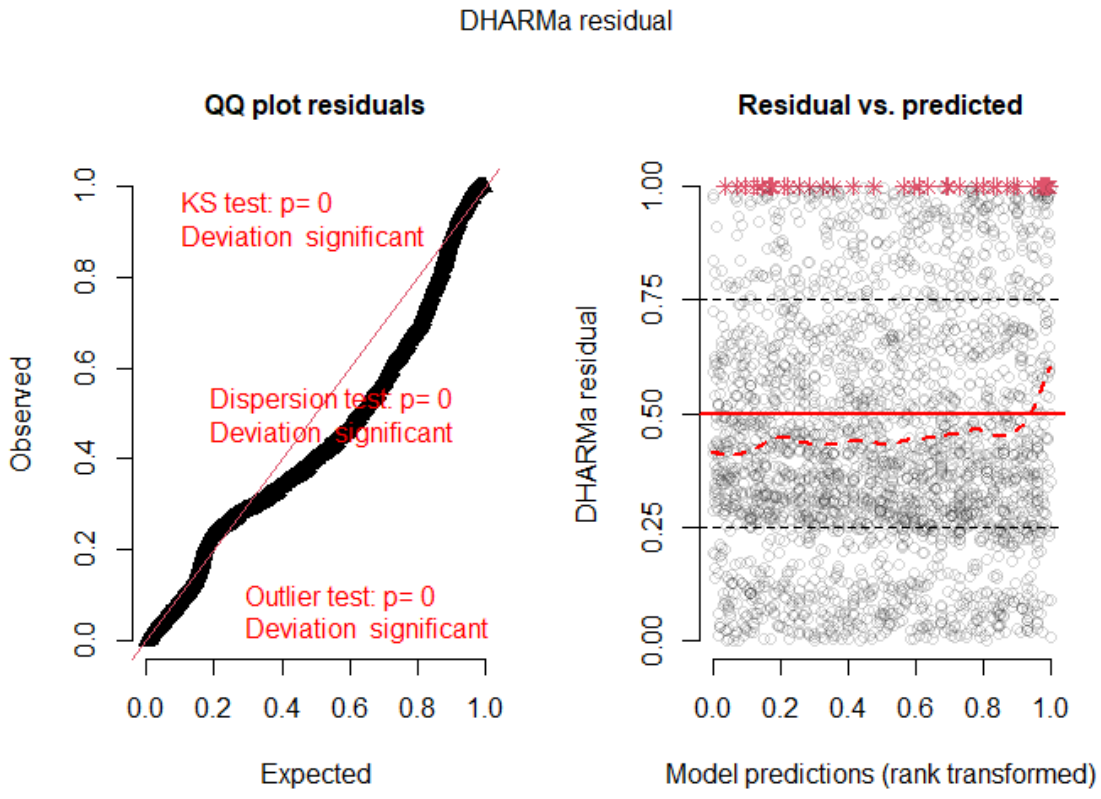
Appendix 10 Cullen and Fery graph showing the distribution of the inbreeding coefficient, F_{ROH}.



Appendix 11 Number of individuals in each stage with F_{ROH} = 0 and F_{ROH} >0.

Stages	No. of individuals	No. of Inds. (F_{ROH} = 0)	No. of Inds. (F_{ROH} >0)	% of Inds. (F_{ROH} = 0)	% of Inds. (F_{ROH} > 0)
Nestling	4785	733	4052	15.319	84.681
Juvenile	3001	456	2545	15.195	84.805
Adult	1728	274	1454	15.856	84.144
Total	9514	1463	8051	15.377	84.623

Appendix 12 Residual plots for zero-inflated beta model extracted by "DHARMa" package.



Appendix 13 The detailed process of examining the distribution of data.

To understand the distribution of the data i.e. inbreeding coefficient. I used the descdist function from the package "fitdistrplus" which helps to map the distribution followed by data (Muller et al., 2023). Upon checking the distribution of F_{ROH}, it showed it lay in the beta family range Appendix 10. But it was quite strange theoretically, beta distribution lies between the open interval of (0, 1) i.e. $0<x<1$ but F_{ROH} is in the close interval [0,1] with 15% data having a value of 0 indicating non-inbred individuals. Appendix 11 shows the distribution of non-inbred in each stage.

As my data contained many zeros and very close to 0 values, I used a zero-inflated beta model to see changes in inbreeding levels in different stages or ages. I fitted the model for the zero-inflation and non-zero parts separately through function glmmTMB from package "glmmTMB" and used the "DHARMa" package to check the model diagnostic (Hartig, 2022) Appendix 12. However, the model diagnostic was too bad, showing all tests were insignificant indicating the model failed to meet the assumptions. This model was also fitted with a different transformed version of F_{ROH} but there was not much progress.

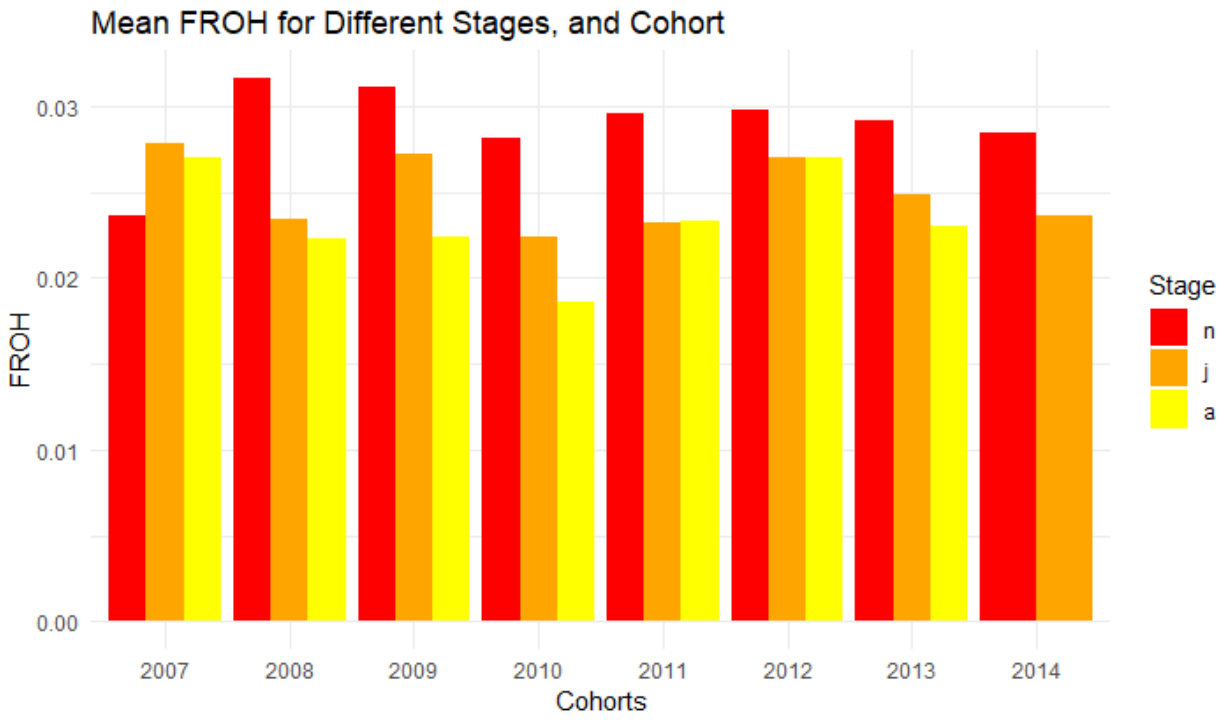
Then again, I tried to understand the distribution of data. As it already showed the beta family from a test of the "fitdistrplus" package, I tried the reverse process. For this, I fitted the beta distribution to the data from function fitdist from package "fitdistrplus" to fit a probability distribution to a dataset using maximum likelihood estimation followed by checking the diagnostic plots Appendix 19. The diagnostic plots do not seem to fit the beta distribution properly. Then, I used one of the goodness of fit tests i.e. the Anderson-Darling test by function Ad.test by specifying to fit beta distribution to data to see if the data followed beta distribution even though this test is especially for checking normality. It rejected the null hypothesis indicating that it did not follow beta distribution. Thus, the test from "fitdistrplus" and the Anderson-Darling test were in opposite directions. I followed the same tests for different values of transformed F_{ROH} including adding a tiny constant of 0.00001 to make F_{ROH} positive. Still, none of the transformed F_{ROH} seemed to follow the beta distribution in both "fitdistrplus" and Anderson-Darling tests.

As the data was heavily right-tailed, I tried this process for some distributions that are supposed to have heavier right-skewed tails like gamma, lognormal, and Weibull. I fitted each distribution to the data, the plot of which is bad compared to fitting beta distribution and goodness of fit test through the Anderson-Darling test also rejected that data follows these distributions. Thus, I could not conclude the distribution because of a limited understanding of the data. Therefore, the permutation test was used which is robust to violation of assumptions of normality.

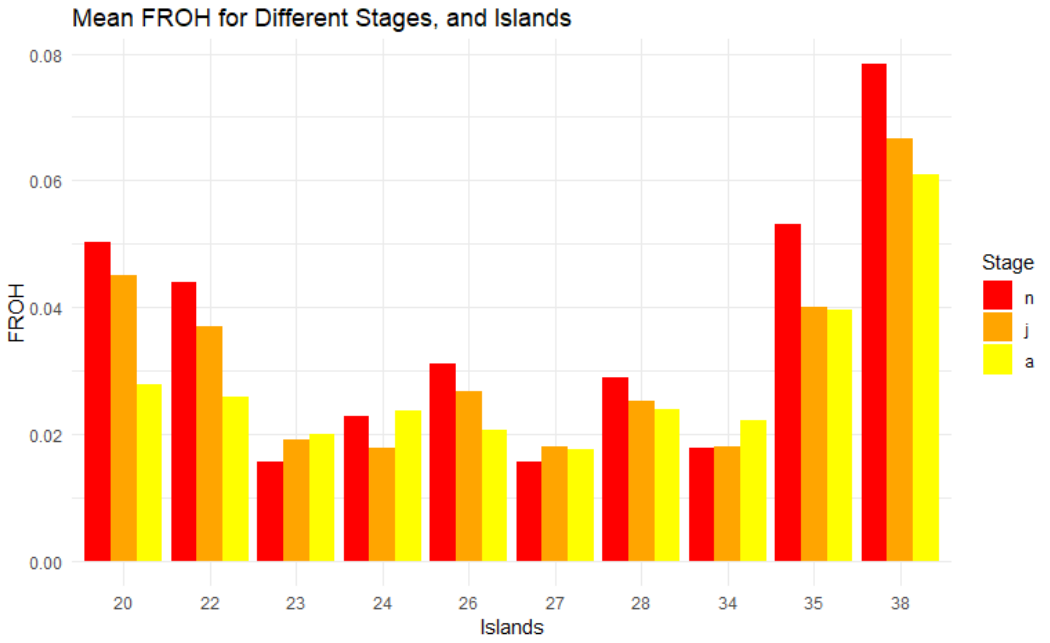
Appendix 14 Sample size and descriptive statistics of inbreeding data for different stages, and means are given with ±1 SD.

Stages	No. of individuals	Mean F_{ROH}	Median F_{ROH}	Range F_{ROH}
Nestling	4785	0.0287(±0.049)	0.0109	0 - 0.4002
Juvenile	3001	0.0246(±0.041)	0.0101	0 - 0.4190
Adult	1728	0.0230(±0.037)	0.0097	0 - 0.3814
Total	9514	0.0264(±0.045)	0.0104	0 - 0.4190

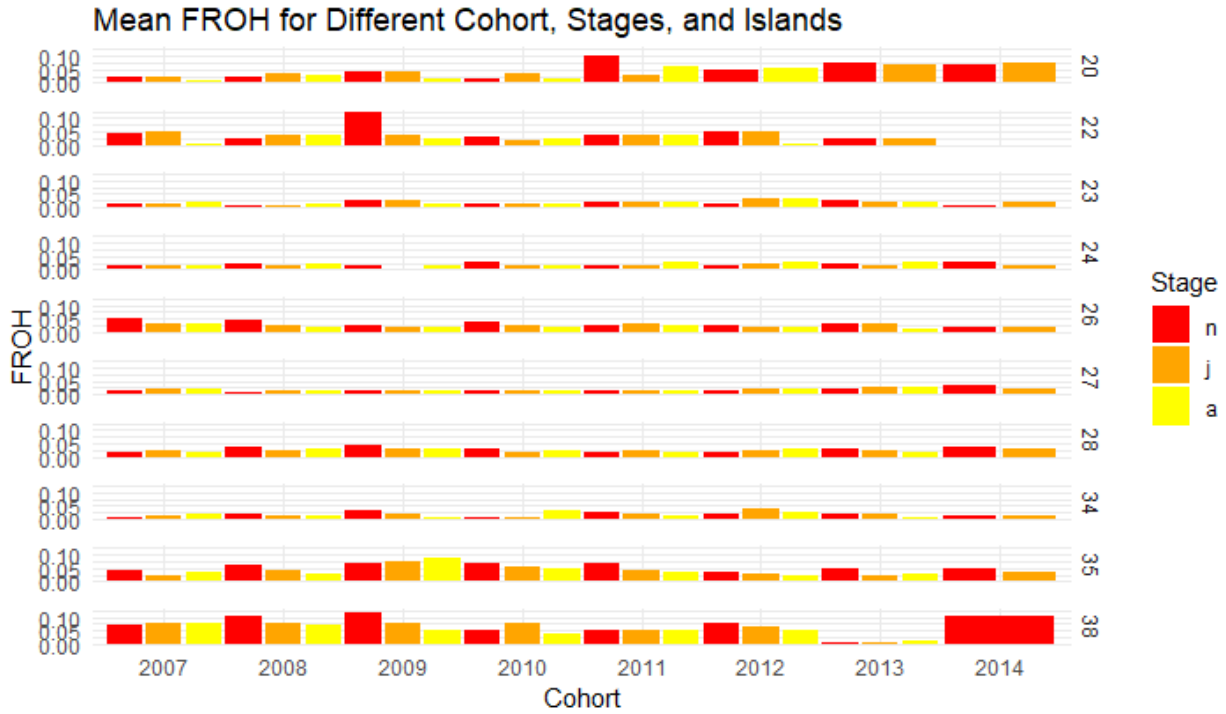
Appendix 15 Mean inbreeding level in different life stages and cohorts.



Appendix 16 Mean inbreeding level for different life stages and islands.



Appendix 17 Combined mean F_{ROH} for stages, islands, and cohorts.

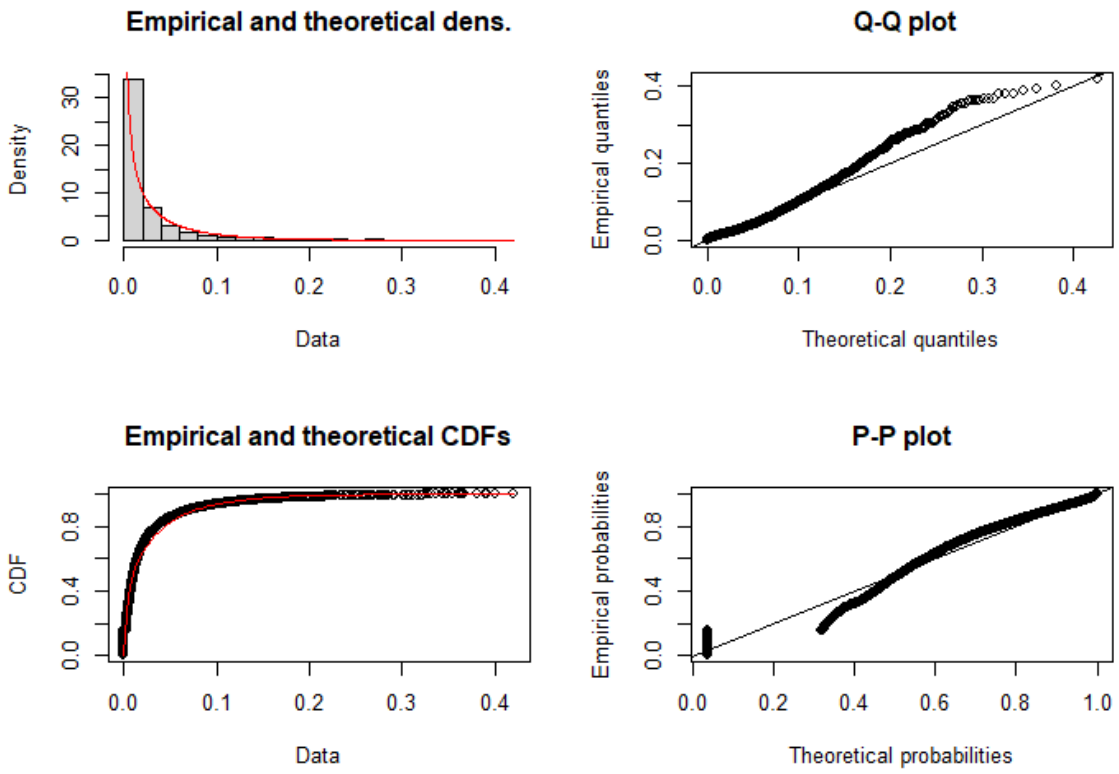


Appendix 18 Parameter estimates of model analyzing how inbreeding coefficient changes with age within nestling analysis.

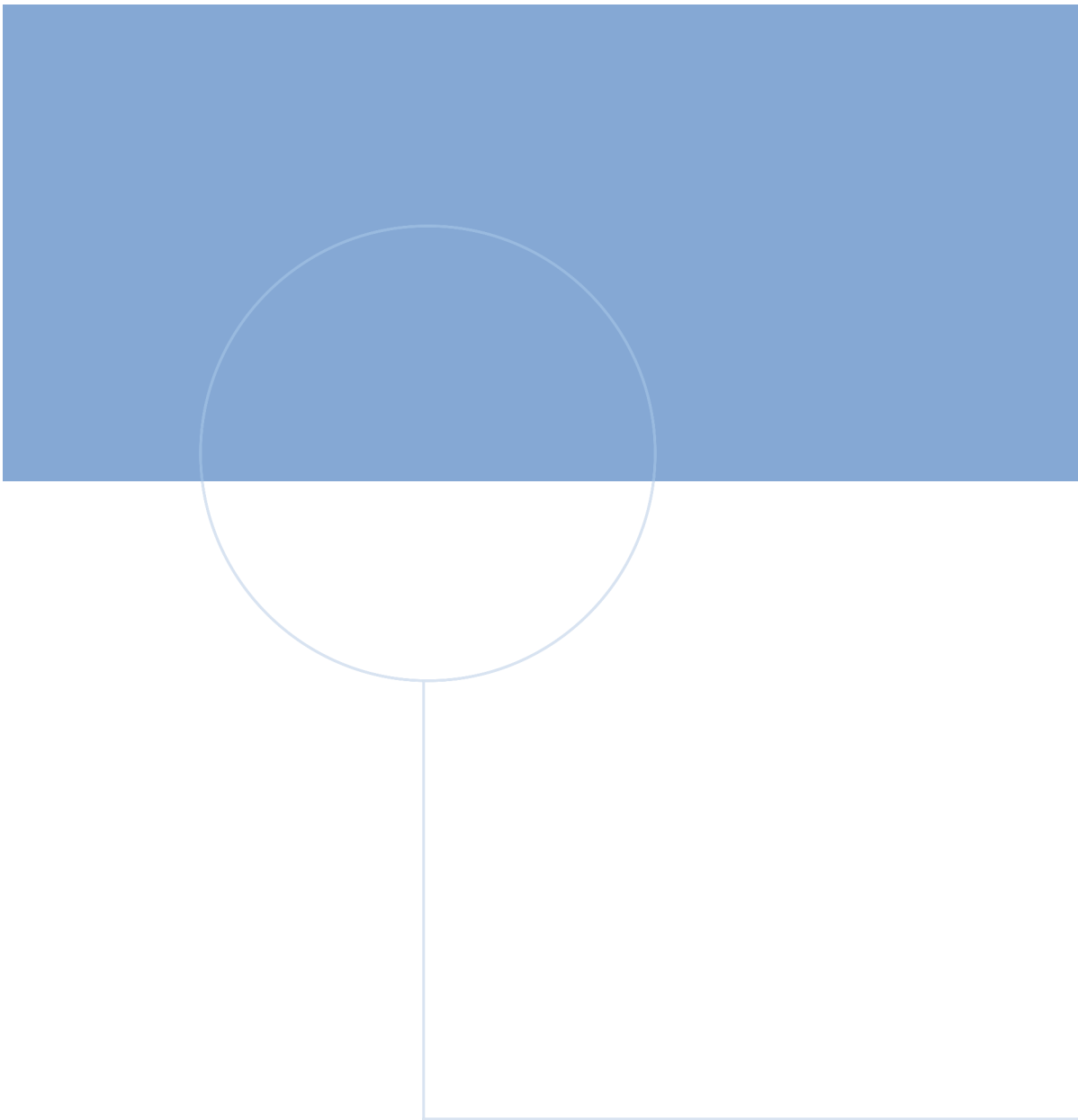
		Confidence interval			
Parameter	Estimate	2.50%	95%	Std. Error	Pr(>|t|)
(Intercept)	0.01239	0.00694	0.03173	0.00990	NA
Nestling age	0.00600	0.00264	0.00936	0.00172	0.0000***
I(Nestling_age^2)	-0.00035	0.00054	0.00016	0.00010	0.0000***
Sex(M)	0.00339	0.00545	0.01223	0.00451	0.0024***
Nestling age: Sex(M)	-0.00046	0.00140	0.00048	0.00048	0.0001***

Note: Parameter estimates, and p-values are from permutation test and confidence interval and standard error from basic linear mixed effect models, so CI and SE are not reliable.

Appendix 19 Diagnostic plot after fitting the beta distribution to inbreeding level data.



Note: a small constant 0.00001 was added to F_{ROH} to make it positive or non-zero as it was not possible to fit distribution with zero values.



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