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Genomic signatures of inbreeding depression for a threatened Aotearoa New Zealand passerine

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Abstract

For small and isolated populations, the increased chance of mating between related individuals can result in a substantial reduction in individual and population fitness. Despite the increasing availability of genomic data to measure inbreeding accurately across the genome, inbreeding depression studies for threatened species are still scarce due to the difficulty of measuring fitness in the wild. Here, we investigate inbreeding and inbreeding depression for the extensively monitored Tiritiri Mātangi island population of a threatened Aotearoa New Zealand passerine, the hihi (*Notiomystis cincta*). First, using a custom 45 k single nucleotide polymorphism (SNP) array, we explore genomic inbreeding patterns by inferring homozygous segments across the genome. Although all individuals have similar levels of ancient inbreeding, highly inbred individuals are affected by recent inbreeding, which can probably be explained by bottleneck effects such as habitat loss after European arrival and their translocation to the island in the 1990s. Second, we investigate genomic inbreeding effects on fitness, measured as lifetime reproductive success, and its three components, juvenile survival, adult annual survival and annual reproductive success, in 363 hihi. We find that global inbreeding significantly affects juvenile survival but none of the remaining fitness traits. Finally, we employ a genome-wide association approach to test the locus-specific effects of inbreeding on fitness, and identify 13 SNPs significantly associated with lifetime reproductive success. Our findings suggest that inbreeding depression does impact hihi, but at different genomic scales for different traits, and that purging has therefore failed to remove all variants with deleterious effects from this population of conservation concern.

KEYWORDS
conservation genomics, genomic inbreeding, inbreeding depression, *Notiomystis cincta*, runs of homozygosity, SNP array

1 | INTRODUCTION

Globally, the erosion of genetic variation and inbreeding depression are two of the main consequences that species of conservation concern, with small isolated populations, are facing (Hoffmann et al., 2017). Inbreeding increases the chances of recessive deleterious alleles being inherited from both parents, which can result in inbreeding depression, a decrease in fitness associated with being inbred (Howard et al., 2017). Although strongly deleterious alleles are thought to be effectively purged from a population over time (Mathur & DeWoody, 2021; Robinson et al., 2018; Xue et al., 2015), the true mutational load remains difficult to quantify (Bosse et al., 2019). Furthermore, inbreeding depression can still manifest in both small and large populations, from the combined effects of many small- to moderate-effect deleterious alleles (Ceballos et al., 2020; Grossen et al., 2020; Robinson et al., 2016). Being recessive, these deleterious alleles can reach reasonable frequencies due to genetic drift, particularly in small populations that have recently undergone a bottleneck event. Along with increased mating between relatives, these populations can transition from carrying a "masked" (i.e., the vast majority of deleterious recessive alleles are carried by heterozygotes) to a "realized" genetic load (i.e., high frequency or even fixation of deleterious recessive variants as an outcome of many individuals becoming homozygous) over time (Bertorelle et al., 2022; Mathur & DeWoody, 2021). Inbreeding depression is readily observed in many wild populations, with negative effects on lifetime breeding success (Huisman et al., 2016), reduced annual survival (Bérénos et al., 2016) and increased mortality at early stages of development (Hedrick & Garcia-Dorado, 2016). In birds, inbreeding can also have severe effects on several traits related to reproduction and survival (Chen et al., 2016; Harrisson et al., 2019; Keller & Waller, 2002), with inbred individuals being less likely to survive, producing fewer offspring and harbouring shorter telomeres that have been associated with poor health and fitness (Niskanen et al., 2020; Pepke et al., 2022). Although inbreeding depression does not necessarily inhibit population growth and recovery (Johnson et al., 2011), it is important to consider when performing population viability analysis in a species management context (Reed et al., 2002) and especially when estimating species extinction risk (Trask et al., 2021; with topic further reviewed in Curik et al., 2017; Hedrick et al., 2017; Howard et al., 2017; Kardos et al., 2016).

In recent decades, disciplines such as animal breeding, conservation and evolutionary genetics have moved from pedigree-based inbreeding estimates (F_{PED}), which capture expected inbreeding levels where full pedigree information is available, to genetic-based inbreeding estimates, which capture realized inbreeding and hence are more accurate than pedigree measures when sufficient numbers of markers are used (Kardos et al., 2015). While initial heterozygosity–fitness studies utilized small sets of markers (Chapman et al., 2009), the rapid uptake of sequencing technologies and genomic tools into the fields of conservation genetics and molecular ecology has enabled heterozygosity and inbreeding to be measured from genome-wide panels of single-nucleotide polymorphisms (SNPs; Huisman et al., 2016; Segelbacher et al., 2021). Furthermore, dense panels of SNPs mapped onto genome assemblies have allowed regions of runs of homozygosity (ROH) across the genome to be identified. These ROH are commonly assumed to reflect sharing of that region from a common ancestor (i.e., be identical by descent), with longer ROH reflecting more recent inbreeding events (Paul et al., 2021). When the sum of the lengths of all ROH are divided by the total autosomal genome size (Hedrick & Garcia-Dorado, 2016), a global (i.e., whole-genome) inbreeding coefficient F_{ROH} can be inferred. These inbreeding coefficients have been shown to be a powerful and accurate tool to describe inbreeding and detect inbreeding depression, especially if a high-quality genome assembly is available (Caballero et al., 2021; Keller et al., 2011; Zilko et al., 2020), and are being increasingly reported (Foote et al., 2021; Grossen et al., 2018; Hedrick et al., 2017; Kyriazis et al., 2022; Nietlisbach et al., 2019).

1.1 | Global vs. regional inbreeding

The availability of genome-wide data enables the investigation of region-specific inbreeding patterns in addition to whole-genome inbreeding coefficients (Howard et al., 2017). This is valuable because focusing solely on the global (whole-genome) inbreeding level might mask some of the variation and effects of region-specific inbreeding and may therefore only partially explain the underlying genetic basis of inbreeding depression. For example, although a substantial proportion of deleterious homozygous genotypes can be found in long ROH—reflecting more recent inbreeding—(Szpiech et al., 2013), it is not expected that all ROH will contain deleterious alleles, and individuals with similar global F_{ROH} values may vary considerably in their realized genetic load depending on which regions, and which alleles, they are homozygous for (Howard et al., 2017). Therefore, regional effects of inbreeding on fitness may be masked if only whole-genome inbreeding is correlated with fitness (Huisman et al., 2016).

To date, several studies have estimated genomic inbreeding and inbreeding depression in the wild using large genome-wide panels of markers (e.g., Foster et al., 2021; Hoffman et al., 2014; Huisman et al., 2016; Harrisson et al., 2019; Kardos et al., 2018; Ochoa & Gibbs, 2021). There has also been extensive application of global and region-specific inbreeding measures to infer production traits, particularly in agricultural species (Doekes et al., 2021; Pryce et al., 2014). However, to our knowledge, only one study system of a natural population has focused on using a finer scale regional inbreeding approach to test for inbreeding depression. In the first of two studies on this wild Soay sheep system, a genome-wide association was used to test whether a homozygous allele within an ROH correlates with an increase or decrease in fitness, and was able to pinpoint a few specific loci that are responsible for a decrease in fitness trait values (Stoffel et al., 2021b). A second study on the same system tested the efficacy of purging of deleterious alleles by exploring the mutation load in short (old) vs. long (recent) inbred regions across the genome, finding that more recent inbred regions carried higher genetic load (Stoffel et al., 2021a).

1.2 | Measuring inbreeding depression

Individual fitness is a crucial component of evolutionary biology, yet is challenging to quantify appropriately, particularly for wild populations (Alif et al., 2022). While long-term data and pedigrees can provide the opportunity to directly measure fitness as the contribution of an individual to offspring of the next generation (i.e., lifetime breeding success; Willoughby et al., 2019), many wild populations lack such detailed monitoring data spanning the entire lifespan of an individual. Therefore, it is commonly short-term measures, such as annual reproductive success, survival to maturity and lifespan, that are used as a proxy for fitness, although because these are only one component of lifetime fitness they may not reveal the true impact of inbreeding (Alif et al., 2022; Zilko et al., 2020). For example, a recent study of the helmeted honeyeater found that although short-term proxies of fitness such as annual reproductive success reveal only weak signatures of inbreeding depression, the associated lifetime effects were stronger (Harrisson et al., 2019).

Furthermore, inbreeding may affect fitness to varying degrees depending on the individual characteristics and environmental context (Reid et al., 2010; Zilko et al., 2020). For example, environmental heterogeneity has been shown to have an impact on the magnitude of inbreeding depression (Fox & Reed, 2011; Szulkin & Sheldon, 2007). A recent meta-analysis of inbreeding depression studies detected minor differences in the effects of inbreeding depression between sexes, but suggested further research was needed to explain the large remaining amount of unexplained heterogeneity (Vega-Trejo et al., 2022). Interactions between environment-dependent trade-offs, sex and senescence can all play a role in the degree to which inbreeding depression impacts an individual or a population. Hence, it is important to also acknowledge the effects of temporal and spatial variation that a population can be subject to, such as changes in dispersal over time (Chen et al., 2016) or sex-specific effects of inbreeding on reproductive senescence (de Boer et al., 2018). Together with inbreeding, these interactions are likely to have particularly profound impacts for threatened species that may already exist in marginal habitats or in unusually high or low population densities.

1.3 | This study

In this study, we quantify the extent and effects of inbreeding on individual fitness (i.e., lifetime reproductive success, also partitioned into juvenile survival, annual adult survival and annual reproductive success) in a translocated population of the threatened hihi (*Notiomystis cincta*) of Aotearoa New Zealand. We analyse SNP chip data for 363 individuals on Tiritiri Mātangi island (36°36′S, 174°53′E) to quantify individual inbreeding levels, and determine whether very recent, medium-term or ancient inbreeding is responsible for higher individual inbreeding levels. Furthermore, we address whether genome-wide or region-specific inbreeding estimated from ROH can explain significant differences in hihi fitness, and if sex, age or lifespan predict individual reproductive success and survival.

As far as we know, this is one of the first studies to report genome-wide and region-specific inbreeding based on extensive ROH metrics to investigate individual and temporal variation in inbreeding and to expose the effects thereof on fitness traits outside the field of animal breeding (Howard et al., 2017; Stoffel et al., 2021a).

2 | MATERIALS AND METHODS

2.1 | Hihi population monitoring and sampling

Once abundant across the North Island, the endemic threatened hihi of Aotearoa New Zealand can now only be found in a single remnant island population on Te Hauturu-o-Toi (36°12′S, 175°05′E; Figure 1) and seven additional reintroduced populations in pest-free sanctuaries. Tiritiri Mātangi island is the largest of the latter populations, with a 2021 population size of 192 hihi (Parlato et al., 2021). Hihi were first translocated from Te Hauturu-o-Toi to Tiritiri Mātangi in 1995 and since then have been subject to long-term study that includes the microsatellite genotyping of all individuals to establish complete pedigrees and the collection of extensive life history data. All fledglings are banded before they leave the nest, survival is surveyed twice a year, and all reproductive attempts across the island are monitored over an individual's lifetime (Low & Part, 2009). This study originally included confirmed genotype data for 480 hihi from Tiritiri Mātangi (Duntsch et al., 2022). However, although there is no natural migration between hihi sites, 98 of the initial sample pool were translocated from Tiritiri Mātangi to other sanctuaries throughout their lives. To reduce population substructure in the data set, a further 19 birds were excluded either because they had been translocated from Te Hauturu-o-Toi to Tiritiri Mātangi in 2010 or were offspring of a translocated bird. Hence, the final data set for this project investigates the remaining 363 individuals, which were sampled between 2001 and 2015, and for which complete survey and breeding data are available, as all sampled birds had died prior to the start of this study.

2.2 | Genotyping and data filtering

In 2016, 1536 hihi from five different populations were genotyped using a custom 50k SNP array (Lee et al., 2022). This Affymetrix (Thermo Fisher Scientific, USA) array was developed based on the identification of SNPs from de novo assembly of restriction site-associated DNA sequencing (RAD-seq) of 31 individuals from the Te Hauturu-o-Toi and Tiritiri Mātangi populations, and low-coverage whole-genome sequencing of 10 of these birds (three of which were from Tiritiri Mātangi). SNPs were selected for approximately even genome spacing by mapping SNP positions from the highly fragmented assemblies to the zebra finch genome. Of the 58,466 SNPs included on the array, 45,553 markers passed initial quality control

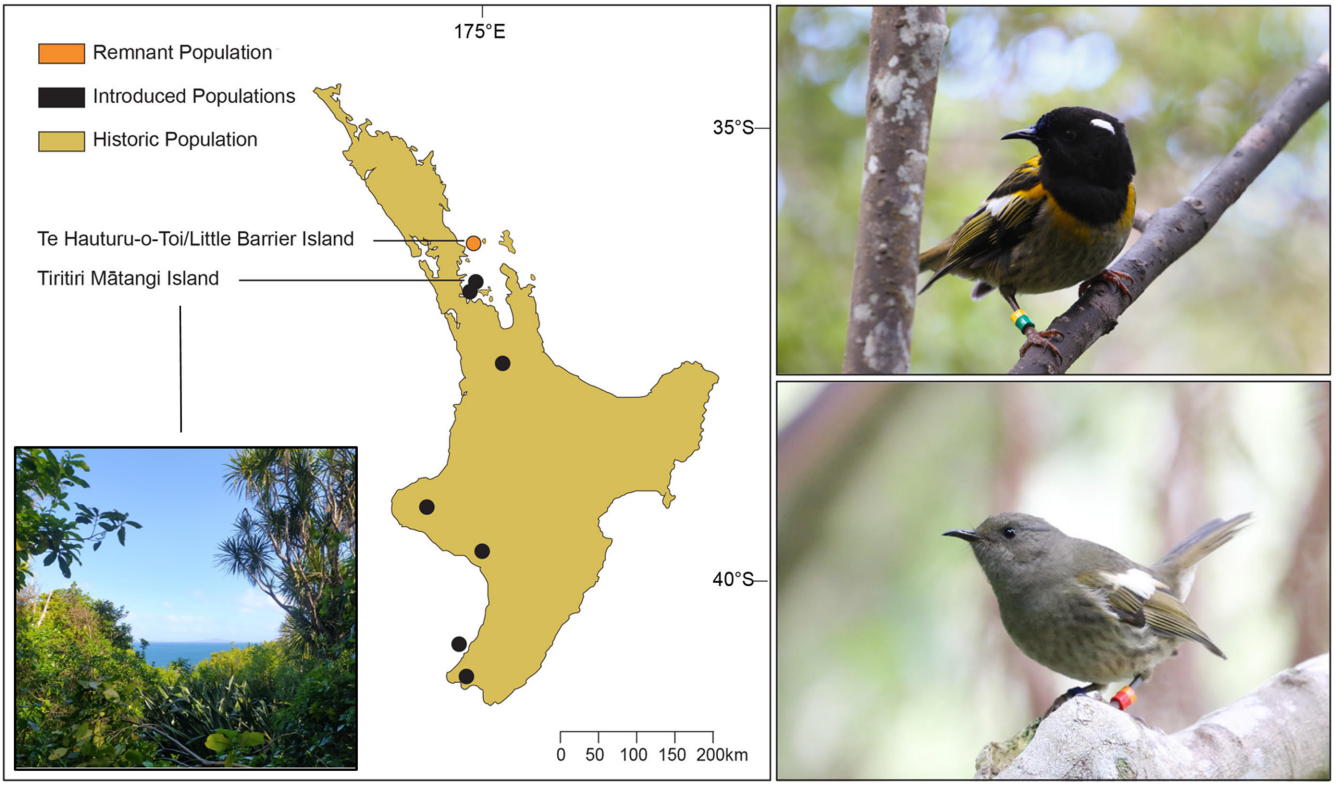

FIGURE 1 A map of the North Island of Aotearoa New Zealand showing the location of the only remnant hihi population on Te Hauturu-o-Toi and of the study population on Tiritiri Mātangi (shown in bottom left photo; taken by Laura Duntsch). Top right: a banded male hihi, taken by Stuart Attwood in Zealandia Sanctuary (the southern-most introduced population) and included with permission. Bottom right: a banded female hihi, taken by Melissa Boardman in Zealandia and included with permission.

metrics in the Axiom Analysis Suite software (polymorphic SNPs with call rate of >95% and well-separated genotype clusters) and PLINK (Purcell et al., 2007) filtering for minor allele frequency and Hardy–Weinberg equilibrium (--maf 0.01, --hwe 1e-20; Duntsch et al., 2021; Lee et al., 2022). The average genotyping rate across all samples is 99.7%, with the mean per-site and per-individual missingness (--lmiss and --imiss in PLINK) not exceeding 0.26%. In 2021, those SNPs were mapped to contigs for a draft hihi reference genome (version NotCin10.4) and those genome contigs were then scaffolded into chromosomes based on homology to zebra finch and the hihi linkage map (*manuscript in preparation*). With sex chromosome positions removed, this resulted in a final data set of 41,195 SNPs with high-confidence assignment to 31 chromosomes. The SNP-containing contigs, scaffolded into chromosomes, cover 86% of the total estimated hihi genome size of 1.06 Gb (A. Whibley et al., unpubl. data).

2.3 | ROH-based inbreeding in hihi

Inbreeding was measured from the SNP data using the hidden Markov model-based approach in the R (R Core Team, 2013) package RZOOROH (Bertrand et al., 2019) that identifies homozygous-by-decent (HBD) segments (Druet & Gautier, 2017) and allows for the estimation of a global inbreeding coefficient. RZOOROH differs from some other inferences of ROH by providing a quantitative probability of an SNP being within an ROH, as well as reporting binary presence/absence, and therefore better captures uncertainty in ROH inference (Bertrand et al., 2019; Druet & Gautier, 2017). In a previous hihi study, we evaluated several models for the number of age-related HBD classes ($K - 1$; the final class represents non-HBD) by varying K from 4 to 13, while allowing for a genotyping error rate of 0.25% (Ferenčaković et al., 2013) and including a new option that improves the partitioning at higher inbreeding levels (layers = TRUE). The 13-class model was determined to be the best fit for hihi (T. Druet, pers. comm.; Duntsch et al., 2021). Hence, the RZOOROH models for this study were fitted with the same rates as our previous study (of 10, 20, 30, 40, 50, 100, 200, 500, 600, 700, 1000, 2000, 2000, where the final 2000 is the non-HBD class). When divided by two, these rates give an approximation of generation time since the most recent common ancestor for the segment falling into this HBD class (Bertrand et al., 2019). For each individual, HBD probabilities were summed over the first 10 HBD classes (excluding HBD classes 11 [rate 1000] and 12 [rate 2000] that did not yield any HBD probabilities for the selected individuals) to give individual inbreeding coefficients (F_{ROH}) for all birds.

We further divided the whole-genome inbreeding level into very recent, middle and ancient inbreeding (F_{rec}, F_{mid}, F_{anc}). Very recent inbreeding F_{rec} includes HBD class 1 (up to 10/2 = 5 generations), middle inbreeding F_{mid} includes the sum across HBD

classes 2–6 (between 5 and 50 generations) and ancient inbreeding F_{anc} includes the sum across HBD classes 7–10 (between 50 and 350 generations). In more detail, F_{rec} includes homozygous segments that originated from inbreeding that happened since the 1990s, or since five generations ago, given the generation time of hihi of ~4.2 years as calculated from the pedigree. This interval therefore includes the year of the first two hihi translocations of 51 birds from Te Hauturu-o-Toi to Tiritiri Mātangi (in 1995 [38 birds] and 1996 [13 birds]; of these 51, only 21 survived to breed; Armstrong et al., 2002) and hence very recent and significant bottleneck events (Brekke et al., 2011). In addition, this first time point was chosen as it is probably recent inbreeding events that have an effect on fitness (Makanjuola et al., 2020). Second, middle inbreeding F_{mid} was defined as the fraction of the whole-genome inbreeding level that captures hihi dynamics after European arrival in Aotearoa New Zealand. More precisely, the F_{mid} interval incorporates inbreeding levels accumulated between the time of the first European settlement in Aotearoa in 1822 and the start of the re-introductions of hihi to other islands and the mainland. Hihi were last seen on the mainland in 1883, and after cats and rats had led to a demise of hihi in the only remnant population on Te Hauturu-o-Toi, the island was declared a predator-free sanctuary in 1980 (Rasch et al., 1996). Lastly, F_{anc} reflects hihi inbreeding more than 50 generations (~200 years) ago and hence is associated with the time before Europeans settled on the North Island, including Māori arrival (~1250; Figure S1). Finally, we identified birds with very high and very low global inbreeding F_{ROH} and investigated the contribution of each of the HBD classes to their overall inbreeding coefficient, and their ROH density on a chromosome level.

We note that a probability-based RZOOROH approach will, on average, yield higher inbreeding values than binary estimates that are offered, for example by PLINK (Meyermans et al., 2020), but our previous work indicates that these values are highly correlated for hihi (Duntsch et al., 2021; also confirmed herein for this set of birds by calculating genome-wide inbreeding from PLINK, Figure S2). As there is considerable variation in recombination rates across the macrochromosomes start-to-end, as well as sex differences in recombination rate for hihi (average recombination is 2.56 centimorgans [cM] Mb^{-1}, A. Whibley et al., unpubl. data), we present results in Mb using default RZOOROH functions and plots. The terms HBD and ROH are used interchangeably, and always refer to aspects of the RZOOROH output.

2.3.1 | ROH density across the population

We measured the average ROH density across the genome for all 363 Tiritiri Mātangi hihi individuals by extracting all HBD segments per chromosome in RZOOROH and estimating mean HBD probabilities of all markers in nonoverlapping 500-kb windows using the R package WINDOWSCANR (Tavares, 2021), following R code provided by Stoffel et al. (2021a). In our data set, the average marker density is 20 SNPs per 500-kb window.

2.4 | Modelling inbreeding depression, age and sex effects

2.4.1 | Important hihi fitness traits

The long-term study of the Tiritiri Mātangi population means that fitness, measured as lifetime reproductive success, is available for all individuals (de Villemereuil et al., 2019). Lifetime reproductive success represents the total number of banded offspring a banded individual had, and hence measures reproductive success across one life cycle, from banding to banding. In determining the impact of both genome-wide and region-specific inbreeding depression, we also partitioned lifetime reproductive success into three components: two annual fitness components—annual reproductive success and adult annual survival—and juvenile survival. The repeated measure of annual reproductive success describes the number of banded offspring a bird had in each breeding season. In addition, adult annual survival is a repeated measure that reflects whether a bird was seen alive in any given year based on the twice-yearly census data. The final trait under investigation is juvenile survival, a trait that describes whether a bird survived for more than two census counts, which roughly equals one calendar year from February to February.

2.4.2 | MCMCGLMM modelling

The effects of whole-genome genomic inbreeding (F_{ROH}), sex and lifespan on lifetime reproductive success (LRS), the effects of inbreeding, age and sex on annual reproductive success (ARS) and the effects of genomic inbreeding and sex on juvenile and adult annual survival (JUS, ADS) were tested using MCMCGLMM (Hadfield, 2010), an R package that fits generalized linear mixed models using Markov chain Monte Carlo techniques. For LRS, fixed predictors included sex (male/female), lifespan (in years) and whole-genome inbreeding F_{ROH} and the interaction between sex and F_{ROH}, while random effects included the breeding season in which a bird was born (birth cohort) and the mother. LRS included lifespan to account for year-to-year stochasticity in survival rates and after confirming no significant impact of inbreeding on lifespan (see Table S2c). LRS was fitted with a Zero inflated Poisson error distribution following de Villemereuil et al. (2019). For ARS, fixed effects of sex, F_{ROH} and age^2 (to reflect observed senescence in reproductive success) and their interactions were fitted, along with random effects of birth cohort, mother, ID and year of measurement. ARS was fitted with a Poisson error distribution. Finally, both ADS and JUS were fitted with interacting fixed effects of F_{ROH} and sex, random effects of birth cohort, mother and ID, and a binomial error distribution ("threshold" family in MCMCGLMM). Furthermore, the ADS model random effects also included the year. Fixed and random components for LRS were included based on previous model selection for a larger LRS data set (de Villemereuil et al., 2019) and were slightly modified for the other traits based on our biological understanding of the species.

We also fitted additional models with the separated inbreeding values (F_{rec}, F_{mid}, F_{anc}), as very recent and middle inbreeding (F_{rec} and

F_{mid}) seemed to contribute most to inbreeding levels in highly inbred birds. All Bayesian models were run for 500,000 iterations after a burn-in period of 3000, sampling every 20th output from the chain, a setting that resulted in a high minimal effective sample size for almost all fixed (>20,000) and random (>5000) effects. Convergence was checked graphically and with the Heidelberger and Welch convergence test using the CODA R package (Heidelberger & Welch, 1981; Plummer et al., 2006). We also fitted all models above without interaction terms, and models of additive vs. interaction fixed effects were compared with the deviance information criterion (DIC).

2.5 | ROH genome wide association scan (GWAS)

We also used the list of identified HBD segments larger than 300 kb from the RZOOROH analysis to test for association between an allele of an SNP being in an ROH and hihi fitness. RZOOROH categorizes regions as being HBD when the HBD probability is >.99 (Bertrand et al., 2019). Following the framework of Stoffel et al. (2021a), for each SNP we fitted a mixed model of association with fixed effects for each of the two SNP alleles and whether they were homozygous and in an ROH or not. The resulting *p*-values for the two predictors at each locus indicate whether an SNP in an ROH for specific allele is significantly associated with the trait (i.e., lifetime reproductive success, annual reproductive success, juvenile survival or adult survival). In addition to the two SNP allele effects, the top seven principal components of the variance-standardized additive relationship matrix (PC1-7; see Stoffel et al., 2021a) were fitted as fixed effects, in lieu of an additive genetic effect (although we note that these additive genetic effects are very small for hihi; see de Villemereuil et al., 2019). The remaining fixed and random predictors were included as selected from the best models from the above MCMCGLMM analysis. Mixed models were fitted with the glmer function from the LME4 package (Bates et al., 2014). Both LRS and ARS glmer models were run with a Poisson error distribution and the ADS and JUS models were run using the binomial distribution (de Villemereuil et al., 2019). The conservative Bonferroni-corrected threshold for genome-wide significance was calculated using the common significance value *p* of .05 in concordance with our previous GWAS analyses (Duntsch et al., 2020). All R scripts and models regarding this mapping of inbreeding depression are modified from Stoffel et al. (2021a) unless otherwise indicated, and can be found in the data availability section of their publication together with a detailed description of their methods.

3 | RESULTS

3.1 | ROH-based inbreeding in hihi

Inbreeding was measured using the R package RZOOROH that identifies homozygous-by-decent segments (HBD; ROH). The probabilities of belonging to each of the HBD classes were summed across the genome to estimate the global genomic inbreeding coefficient. We found that hihi on Tiritiri Mātangi have relatively high average inbreeding levels (mean F_{ROH} ~0.29, Figure 2a; Figure S1 and Table S1). In total, we identified 89,061 HBD segments across all 363 hihi (~245 per individual), with the majority of segments smaller than 300 kb and in higher HBD classes (Figure S3). The largest HBD segment was more than 59 Mb long, the average length per segment was 0.9 Mb and the mean number of SNPs per identified HBD segment was 44. When only including segments longer than 300 kb, the average segment size is 1.4 Mb. Although higher on average, the global total genomic inbreeding from RZOOROH was strongly correlated with the measure from PLINK (PLINK average = 0.24, correlation = .99; Figure S2). Middle inbreeding (between five and 50 generations) shows the highest positive correlation with total inbreeding, while ancient inbreeding was weakly negatively correlated with global inbreeding (Figure S4).

In all highly inbred birds we find that most markers, when homozygous, have a high probability of being a homozygous segment in the first two HBD classes (blue bars in Figure 2b). This suggests the most recent common ancestors of these highly inbred birds have lived within the last 10 generations. All individuals show similar contributions to inbreeding from generations further in the past (dark orange bars in Figure 2b). In addition, birds with higher global inbreeding F_{ROH} also display more and longer HBD segments on the chromosome level (Figure S5).

3.1.1 | ROH density across the population

For the population as a whole, we calculated the mean HBD probability in nonoverlapping 500-kb windows across the genome. The mean window HBD probability never exceeded 50%, suggesting high variation in regional inbreeding between individuals (Figure S6). This means that no large stretches of the genome are strongly affected by inbreeding in all individuals, nor are there many genomic regions that do not have some degree of homozygosity.

3.1.2 | Population averages of lifespan and reproduction

The average lifespan for the hihi presented in this study is 3.16 years (females: 2.9 years; males: 3.3 years) across all 363 birds, but 4.4 years (females: 4.2 years; males: 4.5 years) when only including birds that survive past their first year. The average total viable offspring number per individual equals 1.85 chicks (females: 2.0; males: 1.8) across all 363 birds but increases to 3.9 chicks when only taking breeding individuals into account. In our sample pool, there is no significant difference between males and females for lifespan (Welch two-sample *t* test, $p = .1448$) or total offspring numbers (Welch two-sample *t* test, $p = .4634$). In contrast, a one-way ANOVA revealed significant differences between birth cohorts for lifespan ($p = 7.49 \times 10^{-9}$; effect size 0.161) and total offspring numbers ($p = 2 \times 10^{-16}$, effect size 0.264), with more recent hihi cohorts producing fewer offspring and having lower lifespans.

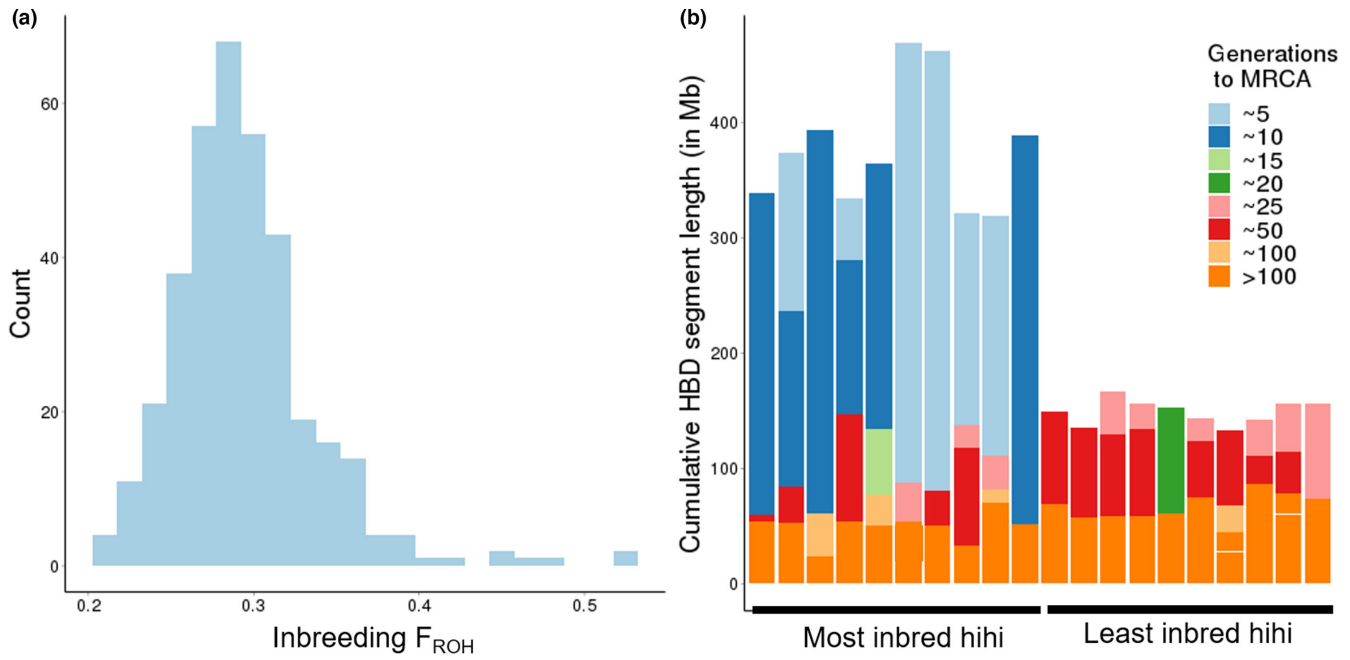

FIGURE 2 (a) Distribution of global (whole-genome) genomic inbreeding values F_{ROH} in the 363 Tiritiri Mātangi individuals as calculated with RZOOROH. (b) The 10 most inbred hihi (first set of bars) and the 10 least inbred hihi (second set of bars) and the contribution of inbreeding over different timescales to the most recent common ancestor (MRCA) that make up their whole-genome inbreeding coefficient. Very inbred birds have high F_{ROH} because of very recent and recent inbreeding (light and dark blue bars, corresponding to HBD classes 1 and 2), indicating inbreeding within the last 10 generations.

3.2 | Inbreeding depression

3.2.1 | Inbreeding effects on reproductive success and hihi survival

When testing for inbreeding depression in hihi from Tiritiri Mātangi island, we found that individual whole-genome inbreeding F_{ROH} significantly negatively affects juvenile survival (JUS; $p = .032$), but, although also estimated to have a negative effect, does not significantly affect annual reproductive success (ARS; $p = .355$), adult survival (ADS: $p = .534$) or lifetime reproductive success (LRS; $p = .404$; Table 1; Tables S2a, S3 and S4) of an individual. Moreover, juvenile survival is significantly affected by the sex of an individual, with males more likely to survive than females ($p = .029$; Figure S7). The interaction term of sex with inbreeding is also significant ($p = .02$), suggesting that more inbred females are less likely to live past the juvenile stage than less inbred ones, whereas male juvenile survival is not as affected by inbreeding. Furthermore, we found hihi lifespan to be the only predictor significantly associated with lifetime reproductive success (LRS) of an individual hihi, with older birds having higher total offspring numbers than birds that die young ($p = .000$). In our models, only the age of an individual significantly predicts its annual reproductive success (ARS), with both males and females having most of their successful offspring between the ages of 3 and 7 years ($p = .000$; Figure S7). Finally, we detected no significant association of inbreeding or sex with our last fitness component, adult annual survival (ADS; Table 1; Table S4).

We further partitioned global inbreeding into very recent inbreeding since their first translocation to the island (F_{rec}; since 1990), middle inbreeding since European arrival to Aotearoa New Zealand (F_{mid}; since 1822) and ancient inbreeding (F_{anc}; Figure S1), and tested the effects thereof on all four fitness traits. First, none of the separated inbreeding values have a significant negative effect on lifetime reproductive success (LRS; Table S6), even though the two inbreeding measures of middle inbreeding F_{mid} and ancient inbreeding F_{anc} show a weak negative correlation with LRS (Figure S8). Similarly, annual reproductive success (ARS) is unaffected by the three partitioned inbreeding measures. Lastly, none of the partitioned inbreeding measures predicted hihi juvenile (JUS) or adult annual survival (ADS; Table S6).

3.3 | GWAS

We constructed a mixed model for LRS, ARS, JUS and ADS to test the effect of each SNP position on fitness when in a homozygous region. Mixed models were formulated similarly to the models in the MCMCGLMM analysis above, with an interaction term between sex and inbreeding (Table 1) but the addition of two fixed effects per SNP allowed the fitness effect of each allele being homozygous and in an ROH to be captured.

Even though the MCMCGLMM analysis revealed no significant effect of global inbreeding on our main fitness trait, our genome-wide association for inbreeding revealed that the ROH status of 13 SNPs was significantly negatively associated with lifetime reproductive success (LRS; Figure 3; Figure S9). These SNPs cluster on three different chromosomes, chromosome 2 (two SNPs), chromosome 5 (nine SNPs) and chromosome 12 (two SNPs), and have similarly

Mcmcglmm	Predictor	Post. Mean	Lower 95% CI	Upper 95% CI	pMCMC
LRS ~ F_{ROH} * Sex + Lifespan	F_{ROH}	−2.395	−8.101	3.190	0.404
	Sex	−0.107	−2.132	1.916	0.919
	Lifespan	0.387	0.327	0.450	**0.000**
	F_{ROH}:Sex	−0.598	−7.469	6.389	0.861
ARS ~ F_{ROH} * Sex + Age^2	F_{ROH}	−2.554	−7.899	2.949	0.355
	Sex	0.527	−1.400	2.385	0.578
	Age	0.376	0.320	0.434	**0.000**
	F_{ROH}:Sex	−2.980	−9.552	3.426	0.369
JUS ~ F_{ROH} * Sex	F_{ROH}	−6.964	−13.375	−0.344	**0.032**
	Sex	−2.466	−4.780	−0.245	**0.029**
	F_{ROH}:Sex	8.786	0.949	16.157	**0.021**
ADS ~ F_{ROH} * Sex	F_{ROH}	−0.879	−3.662	1.898	0.534
	Sex	−0.051	−0.983	0.924	0.919
	F_{ROH}:Sex	0.727	−2.578	3.962	0.663

pMCMC; values <0.05 are given in bold.

TABLE 1 MCMCGLMM output for the four Bayesian models testing inbreeding F_{ROH} effects as well as age and sex effects on lifetime reproductive success (LRS; ZiPoisson error structure), annual reproductive success (ARS; Poisson error structure), juvenile survival (JUS; binomial error structure) and annual adult survival (ADS; binomial error structure). An interaction term between inbreeding and sex was included in all models. Post. mean is the posterior mean, while lower and upper credible intervals (CI) are provided along with the probability of the value of the predictor differing from zero. A full table with all model outputs and details on random effects can be found in Tables S2–S5.

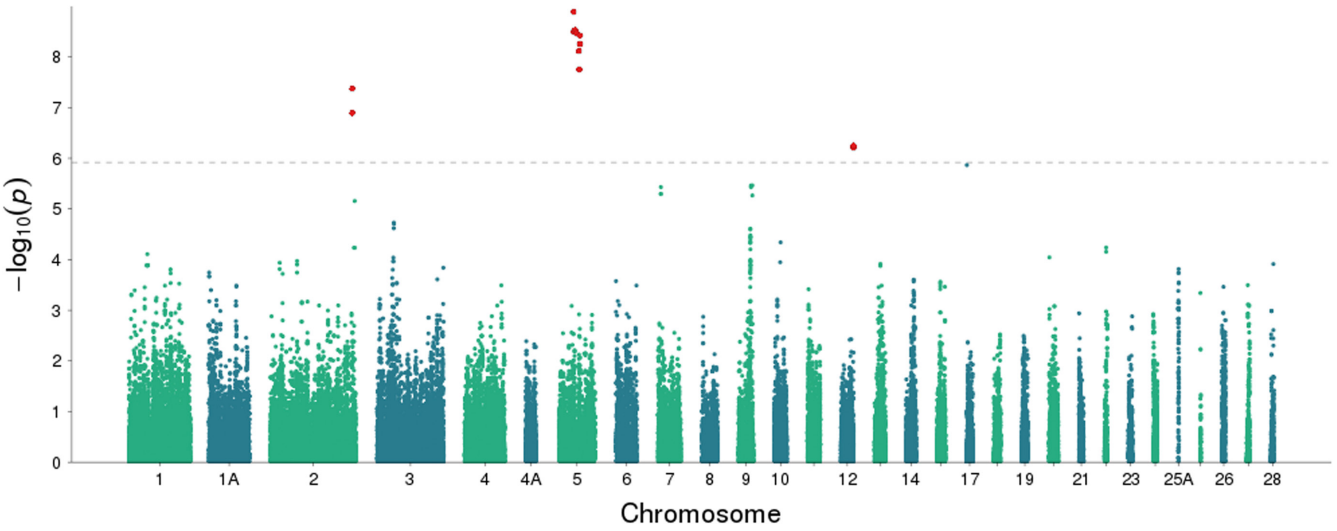

FIGURE 3 Manhattan plot showing that 13 of the SNPs, when homozygous and in a ROH, tested in our interaction model have a significant negative effect on fitness (LRS) in the population of hihi on Tiritiri Mātangi. The dotted line is the conservative Bonferroni-corrected threshold.

large effect sizes (Table S7). In contrast, none of the SNPs when homozygous and in an ROH was significantly associated with annual reproductive success (ARS), in agreement with the lack of effect of whole-genome inbreeding on that trait (Figure S10). Similarly, we did not detect any significant association of the ROH status of SNPs with juvenile or adult annual survival, with all *p*-value estimates well below the conservative Bonferroni significance threshold (Figure S10, qq-plots in Figure S11).

4 | DISCUSSION

Novel genetic tools can help us better understand the impact of inbreeding and small population size on the adaptive potential of hihi, a species of conservation concern. Here, we used genomic data to infer individual inbreeding for hihi from Tiritiri Mātangi island and correlated these inbreeding levels with lifetime fitness, as well as its components—annual reproductive success, juvenile survival and adult survival. In sum, we find a significant negative effect of global inbreeding on juvenile survival and local inbreeding effects on lifetime reproductive success for this threatened species.

4.1 | Genome-wide and regional inbreeding

In this study, we find that genome-wide inbreeding levels for the 363 hihi from the Tiritiri Mātangi population are relatively high ($F_{ROH}^{RZOOROH}$ =0.29, F_{ROH}^{PLINK} =0.24) when compared to inbreeding

levels in all remaining individuals of another threatened Aotearoa New Zealand bird species, the kākāpō (*Strigops habroptilus*; F_{ROH}^{PLINK} =0.18; using 12,241 SNPs; Foster et al., 2021). Hihi ROH-based inbreeding is also much higher than in a population of a US endemic avian species of conservation concern, the Florida scrub jay (*Aphelocoma coerulescens*; F_{ROH}^{PLINK} =0.012; using 11,737 SNPs; Nguyen et al., 2022), which suggests that expected inbreeding levels in threatened species will vary depending on extant and past population size, recent demographic history, life history, natural dispersal and management strategies (de Kort et al., 2021). Furthermore, we were able to show that many of the hihi individuals with very high genome-wide inbreeding levels have experienced inbreeding recently, within the last five generations, which coincides with the timing of the original translocation of 21 surviving birds from Te Hauturu-o-Toi in the mid-1990s as well as subsequent reinforcements to Tiritiri Mātangi (Brekke et al., 2011; de Villemereuil et al., 2019). Many highly inbred individuals also display higher inbreeding levels due to events towards the middle of the last century (~10 generations ago), when hihi were long gone from the mainland. During that time, a drastic decline of hihi in the last remnant population on Te Hauturu-o-Toi due to rat and cat predation was recorded, presenting yet another bottleneck event that hihi underwent in the last century (Rasch et al., 1996). Together, our findings of enhanced inbreeding levels on Tiritiri Mātangi agree with previous studies detecting fewer polymorphic sites in the Tiritiri Mātangi population compared to the remnant Te Hauturu-o-Toi population, albeit these studies had small sample sizes (Brekke et al., 2011; de Villemereuil et al., 2019). In addition, the ROH analysis revealed that, although the majority of ROH are small, some regions of homozygosity are very large, spanning up to 59 Mb (60% of chromosome 1). This is much longer than, for example, the longest run of homozygosity (17.5 Mb) in a large-scale collared flycatcher (*Ficedula albicollis*) study using 104 resequenced genomes (Kardos et al., 2017), while more comparable to highly inbred kākāpō displaying ROH sizes of up to 75.61 Mb (Foster et al., 2021). However, on a population scale, we detected notable variation in inbreeding and inbreeding patterns between individuals, and no genomic region was inbred in more than 50% of individuals. Similar findings were reported in a farmed rainbow trout population, with high variation in individual inbreeding and chromosomal inbreeding levels along the genome (Paul et al., 2021).

4.2 | The fitness consequences of inbreeding

Given the mostly low effective population sizes, close relationships and variable selection pressures in species of conservation concern (Ceballos et al., 2018), understanding the direct fitness consequences of inbreeding is a topic of high priority for threatened species such as hihi. We know that the number of loci genotyped with the custom hihi SNP array is sufficient to detect ROH in over 88% of the autosomal genome, and hence provide enough power to detect inbreeding–fitness correlations in this population (Duntsch et al., 2021; Miller & Coltman, 2014). When correlating whole-genome inbreeding levels with four important fitness traits for individuals from the Tiritiri Mātangi population, we were able to detect a significant negative effect of global inbreeding on hihi juvenile survival. Hence this study is a valuable addition to previous inbreeding depression studies investigating the effects of inbreeding on traits related to reproduction and survival in small populations (Billing et al., 2012; de Boer et al., 2018; Flanagan et al., 2021; Foote et al., 2021; Hansson & Westerberg, 2002; Hedrick & Garcia-Dorado, 2016; Hoeck et al., 2015; Hoffman et al., 2014; Howard et al., 2017; Kardos et al., 2018; Keller & Waller, 2002; Kennedy et al., 2014; Khan et al., 2021; Vega-Trejo et al., 2022; Willoughby et al., 2019).

Our study suggests that global inbreeding has a significant negative effect on hihi juvenile survival, where less inbred individuals are more likely to survive past the juvenile stage than highly inbred birds. Furthermore, the effect of inbreeding on juvenile survival appears to differ between the sexes, with inbred females showing higher mortality within their first year than inbred males. In the past, inbreeding has been shown to have greater impact on male compared to female hihi survival at early stages of development based on microsatellite markers (Brekke et al., 2011). Furthermore, a male bias in mortality has been observed in the population at the embryo development and nestling stage (F. Morland, pers. comm.), whereas our study suggests higher inbred female mortality later in their juvenile life. Therefore, inbreeding may impact hihi at slightly different stages of development between males and females. Overall, this implies that highly inbred hihi individuals may be removed from the Tiritiri Mātangi population before they can reproduce, a scenario that would see a decrease in the overall levels of inbreeding in the breeding part of the population. In addition, we show that annual reproductive success is age-dependent in the hihi population, with birds younger than 3 years and older than 7 years showing lower annual offspring numbers, agreeing with previous findings of senescence in hihi (Low & Part, 2009). Our results are supported by various studies that investigated sex-specific inbreeding depression effects in wild populations (Billing et al., 2012), reporting faster reproductive senescence for inbred females but not for males and different relationships between inbreeding, age and disease susceptibility for males and females (Benton et al., 2018). In the future, it will remain important to consider factors such as sex and senescence when evaluating the genetic health of a population, as inbreeding depression across different life stages and sexes remains to be fully understood (Trask et al., 2021; Vega-Trejo et al., 2022).

The fact that we could not detect a significant effect of individual whole-genome inbreeding on lifetime reproductive success, annual reproductive success and annual adult survival suggests that genome-wide inbreeding may not fully capture the inbreeding load of individuals, there is considerable variation in the impacts of inbreeding over time, and/or we may lack power to detect a significant impact of inbreeding on these traits. While we detected a significant effect of inbreeding on some but not all chosen fitness proxies on Tiritiri Mātangi, this does not prove the absence of an inbreeding effect on other traits (Altman & Bland, 1995), especially with regard to the large credible intervals. We also suspect that the population is experiencing

weaker selection against deleterious variants and can tolerate higher levels of inbreeding as long as supplementary feeding is provided and environmental conditions are ideal (Armstrong et al., 2007; Chauvenet et al., 2012; Ewen et al., 2015). It is also worth noting that the population size of hihi on the island has increased steadily over the course of this study, with a total population size of 100 birds in 2004, to 170 individuals in 2015, potentially increasing competition for territories, resources and mates, while reducing chances to mate with a close relative. However, we are aware that multiple factors can contribute to a lack of power to detect whole-genome inbreeding effects on our main fitness trait. While we included the most obvious predictors such as sex and age of the individual in our mixed models, and fitted birth cohort, year, mother and ID as random effects, additional environmental effects that have been unaccounted for might in fact be the main drivers of fitness in adult birds. Those factors include but are not limited to fluctuations in the average temperature of each year, droughts and heavy rainfall, natural food availability and phenology, and microclimate effects and hihi density per individual territory. Moreover, the most important contributors to fitness may vary depending on the population across the North Island (Rutschmann et al., 2020). Other hihi populations, such as Zealandia sanctuary, Wellington, are smaller, surrounded by different types of flora and coexist with different avian species than Tiritiri Mātangi, and hence could be affected differently by inbreeding. Future studies may also measure genomic inbreeding depression for additional components and key adaptive traits such as body size, mating success, breeding strategy, traits measured at different development stages or annual fitness in the first year of life (Alif et al., 2022).

4.3 | Genome-wide association of inbreeding depression

High variation in inbreeding landscapes between individuals might mask the effect of regional inbreeding on important hihi fitness traits when the overall genome-wide inbreeding effect is non-significant (Paul et al., 2021; Slate et al., 2004). We conducted a genome-wide association study for inbreeding depression by using the ROH status of an SNP as a predictor of fitness, in order to further investigate inbreeding effects on our four measured fitness components. This regional genomic approach was able to detect 13 loci with negative effects on lifetime reproductive success of hihi, a finding that would have gone unnoticed using a genome-wide inbreeding approach alone. These SNPs are located on chromosome 2 (two SNPs mapping to positions 137,385,541 and 137,597,845), a large region across chromosome 5 (nine SNPs mapping to positions 25,343,922–35,850,425) and chromosome 12 (two SNPs mapping to positions 21,012,542 and 21,112,406). Some of these loci are located near (within a few hundred kb upstream and downstream) of protein-coding genes, according to the zebra finch genome annotation (assembly version number bTaeGut1_v1.*p*). Examples are *EHD4* (enables cadherin binding activity) and *SMOC1* (a calcium-binding protein) on chromosome 5 and *EXT1* (exostosin glycosyltransferase) on chromosome 2, a putative tumour suppression protein. All of the SNPs with negative effect on fitness represent minor alleles, with their allele frequency ranging from 0.06 to 0.24 (see Table S7), which suggests that these SNPs may be in linkage disequilibrium, with recessive deleterious mutations generating inbreeding depression by appearing in a homozygous state in inbred individuals. It is important to note that some important genomic regions may not be well tagged by the genotyped SNPs; for example, no SNPs were successfully genotyped on microchromosome 16, which is thought to contain the major histocompatibility complex (MHC; Lee et al., 2022).

We did not detect local SNP effects on juvenile survival, which is contrary to our findings of a significant whole-genome inbreeding effect on this trait. Similarly, our genome-wide scan for association between an SNP in an ROH and annual fitness did not reveal any variants that were significantly associated with a reduction in annual offspring numbers or reduced adult annual survival, in agreement with the lack of significant effects of whole-genome inbreeding for these two traits. Overall, it appears that high individual variation in individual F_{ROH} may have masked region-specific inbreeding effects on our main fitness trait, lifetime reproductive success, but that we may have lacked power to detect region-specific effects of inbreeding for the three remaining fitness components in hihi.

4.4 | Hihi conservation genomics

Hihi are extremely vulnerable to all predators and competitors as well as a change in climate. Hence, understanding the genetic architecture of crucial fitness traits in the main source population for translocations of hihi, Tiritiri Mātangi island, can help us understand just how compromised the species' evolutionary capacity is (de Villemereuil et al., 2019; Duntsch et al., 2020). We were able to detect effects of inbreeding depression in hihi on Tiritiri Matangi island, while previous studies have found reduced adaptive potential given a low additive genetic variance of fitness in the species (de Villemereuil et al., 2019; Bonnet et al., 2022). This potentially suggests that the small but nonzero genetic contribution to fitness differences was captured because of the presence of moderately deleterious variants that have failed to be purged from the population, possibly because they were recessive rather than codominant or dominant. These moderate-effect deleterious recessive mutations may have increased in frequency over time due to drift (Hedrick & Garcia-Dorado, 2016) and the relatively high levels of inbreeding have exposed genetic load from these recessive mutations, leading to inbreeding depression. In support of this hypothesis, it is notable that we have detected a significant association of the homozygous state of 13 SNPs with fitness, which would not be expected if inbreeding depression was explained solely by many very small-effect loci (although we acknowledge that the SNP effect sizes are probably overestimated; Slate, 2013). From a species conservation perspective, this indicates that hihi mean fitness is expected to further decrease if inbreeding rates increase in the Tiritiri Matangi population. Future conservation genomic work for

hihi might therefore focus not only on the study of rare adaptive alleles, but more importantly on the detection and mitigation of the accumulation of recessive deleterious mutations in all translocated populations.

While the role of purging on survival and reproductive success has been discussed for many mammalian species with long-term pedigree data (Kyriazis et al., 2022), this kind of insight is still scarce in birds and threatened species in general. That being said, the exact status of hihi genomic vulnerability remains to be assessed by future whole-genome sequencing approaches, which will enable us to take a closer look at the loci and regions contributing to inbreeding depression in hihi discovered in these analyses, alongside an analysis of selection and diversity in the genome. Future simulations will also show whether inbreeding depression is likely to translate into reduced population growth and recovery (Johnson et al., 2011). Our results advocate for and support the move away from only testing the fitness effects of global whole-genome inbreeding values to also investigating the impacts of region-specific inbreeding estimates for a species of conservation concern, as global inbreeding alone might not be a suitable estimation of genetic health in all wild populations. This will more reliably capture the true inbreeding landscape of individuals in small populations and help to recognize, monitor and mitigate the fitness consequences of bottleneck events.

AUTHOR CONTRIBUTIONS

L.D. and A.W.S. designed the research, and L.D. processed and analysed the data and performed the research. A.W. and S.B. led the hihi genome assembly and provided chromosome-level SNP positions. P.d.V. aided with model selection and provided feedback on the GWAS procedure. P.B. developed the microsatellite data set, supervised the genotyping, and performed the pedigree reconstruction. J.G.E. developed the demographic data set and supervised the data collection. L.D. led the writing of the paper, with input from A.W.S. and feedback from A.W., P.d.V., S.B., P.B. and J.G.E. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Supporting methods, results, figures and tables are provided in the Supporting Information. Hihi are of cultural significance to the indigenous people of Aotearoa New Zealand, the Māori, and are considered a taonga (treasured) species whose whakapapa (genealogy) is intricately tied to that of Māori. For this reason, the genotypes and associated metadata for hihi will be made available by request on the recommendation of Ngāti Manuhiri, the iwi (tribe) that affiliates as kaitiaki (guardians) for hihi. To obtain contact details for the iwi, please contact Dr Anna Santure: a.santure@auckland.ac.nz.

BENEFIT SHARING

We consulted with the indigenous community, the iwi (tribe) Ngāti Manuhiri, who are kaitiaki (guardians) for hihi. In the Acknowledgements we acknowledge Ngāti Manuhiri as Mana Whenua and Kaitiaki of Te Hauturu-o-Toi and its taonga, including hihi. Regular hihi updates are provided to Ngāti Manuhiri via the Department of Conservation Hihi Recovery Group reports.

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SUPPORTING INFORMATION

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