

1 GENOMIC SIGNATURES OF INBREEDING DEPRESSION  
2 FOR A THREATENED AOTEAROA NEW ZEALAND  
3 PASSERINE  
4

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## 7 Abstract

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

28 *Keywords: genomic inbreeding; runs of homozygosity; inbreeding depression; SNP array;*  
29 *conservation genomics; Notiomystis cincta*

## 30 Introduction

31 Globally, the erosion of genetic variation and inbreeding depression are two of the main  
32 consequences that species of conservation concern, with small isolated populations, are  
33 facing (Hoffmann et al., 2017). Inbreeding increases the chances of recessive deleterious  
34 alleles being inherited from both parents, which can result in inbreeding depression, a  
35 decrease in fitness associated with being inbred (Howard et al., 2017). Although strongly  
36 deleterious alleles are thought to be effectively purged from a population over time (Xue et  
37 al., 2015; Robinson et al., 2018; Mathur & DeWoody, 2021), the true mutational load remains  
38 difficult to quantify (Bosse et al., 2019). Furthermore, inbreeding depression can still manifest  
39 in both small and large populations, from the combined effects of many small to moderate-  
40 effect deleterious alleles (Robinson et al., 2016; Ceballos et al., 2020; Grossen et al., 2020).  
41 Being recessive, these deleterious alleles can reach reasonable frequencies due to genetic  
42 drift, particularly in small populations that have recently undergone a bottleneck event. Along  
43 with increased mating between relatives, these populations can transition from carrying a  
44 'masked' (i.e., the vast majority of deleterious recessive alleles are carried by heterozygotes)  
45 to a 'realized' genetic load (i.e., high frequency or even fixation of deleterious recessive  
46 variants as an outcome of many individuals becoming homozygous) over time (Mathur &  
47 DeWoody, 2021; Bertorelle et al., 2022). Inbreeding depression is readily observed in many  
48 wild populations, with negative effects on lifetime breeding success (Huisman et al., 2016),  
49 reduced annual survival (Bérénos et al., 2016) and increased mortality at early stages of  
50 development (Hedrick & Garcia-Dorado, 2016). In birds, inbreeding can also have severe  
51 effects on several traits related to reproduction and survival (Keller & Waller, 2002; Chen et  
52 al., 2016; Harrisson et al., 2019), with inbred individuals being less likely to survive,  
53 producing fewer offspring and harbouring shorter telomeres that have been associated with  
54 poor health and fitness (Niskanen et al., 2020; Pepke et al., 2022). Although inbreeding  
55 depression does not necessarily inhibit population growth and recovery (Johnson et al.,  
56 2011), it is important to consider when performing population viability analysis in a species  
57 management context (Reed et al., 2002) and especially when estimating species extinction  
58 risk (Trask et al. 2021; with topic further reviewed in Kardos et al., 2016, Hedrick et al., 2016,  
59 Curik et al., 2017 and Howard et al., 2017).

60 In recent decades, disciplines such as animal breeding, conservation and evolutionary  
61 genetics have moved from pedigree-based inbreeding ( $F_{PED}$ ), which captures expected  
62 inbreeding levels where full pedigree information is available, to genetic-based inbreeding  
63 estimates, which capture realised inbreeding and hence are more accurate than pedigree  
64 measures when sufficient numbers of markers are used (Kardos et al., 2015). While initial  
65 heterozygosity-fitness studies utilised small sets of markers (Chapman et al., 2009), the rapid  
66 uptake of sequencing technologies and genomic tools into the fields of conservation genetics  
67 and molecular ecology has enabled heterozygosity and inbreeding to be measured from  
68 genome-wide panels of single-nucleotide-polymorphisms (SNPs; Huisman et al., 2016;  
69 Segelbacher et al., 2021). Further, dense panels of SNPs mapped onto genome assemblies  
70 have allowed regions of runs of homozygosity (ROH) across the genome to be identified.  
71 These ROH are commonly assumed to reflect sharing of that region from a common  
72 ancestor (i.e., be identical by descent), with longer ROH reflecting more recent inbreeding  
73 events (Paul et al., 2021). When the sum of the lengths of all ROH are divided by the total

74 autosomal genome size (Hedrick & Garcia-Dorado, 2016), a global (i.e., whole-genome)  
75 inbreeding coefficient  $F_{ROH}$  can be inferred. These inbreeding coefficients have been shown  
76 to be a powerful and accurate tool to describe inbreeding and detect inbreeding depression,  
77 especially if a high-quality genome assembly is available (Keller et al., 2011; Zilko et al.,  
78 2020; Caballero et al., 2021), and are being increasingly reported (Hedrick et al., 2017;  
79 Grossen et al., 2018; Nietlisbach et al., 2019; Foote et al., 2021; Kyriazis et al., 2022).

## 80 Global versus regional inbreeding

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

93 To-date, several studies have estimated genomic inbreeding and inbreeding depression in  
94 the wild using large genome-wide panels of markers (e.g. Hoffman et al., 2014; Huisman et  
95 al., 2016; Kardos et al., 2018; Harrisson et al., 2019; Foster et al., 2021; Ochoa & Gibbs,  
96 2021). There has also been extensive application of global and region-specific inbreeding  
97 measures to infer production traits, particularly in agricultural species (Pryce et al., 2014;  
98 Doekes et al., 2021). However, to our knowledge, only one study system of a natural  
99 population has focussed on using a finer-scale regional inbreeding approach to test for  
100 inbreeding depression. In the first of two studies on this wild Soay sheep system, a genome-  
101 wide association was used to test whether a homozygous allele within an ROH correlates  
102 with an increase or decrease in fitness, and was able to pinpoint a few specific loci that are  
103 responsible for a decrease in fitness trait values (Stoffel et al., 2021b). A second study on the  
104 same system tested the efficacy of purging of deleterious alleles by exploring the mutation  
105 load in short (old) versus long (recent) inbred regions across the genome, finding that more  
106 recent inbred regions carried higher genetic load (Stoffel et al., 2021a).

## 107 Measuring inbreeding depression

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

118 reproductive success reveal only weak signatures of inbreeding depression, the associated  
119 lifetime effects were stronger (Harrisson et al., 2019).

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

## 135 This study

136 In this study, we quantify the extent and effects of inbreeding on individual fitness (i.e.,  
137 lifetime reproductive success, juvenile survival, annual adult survival and annual reproductive  
138 success) in a translocated population of the threatened hihi (*Notiomystis cincta*) of Aotearoa  
139 New Zealand. We analyse SNP chip data for 363 individuals on Tiritiri Mātangi island  
140 (36°36'S, 174°53'E) to quantify individual inbreeding levels, and determine whether very  
141 recent, medium-term or ancient inbreeding is responsible for higher individual inbreeding  
142 levels. Furthermore, we address whether genome-wide or region-specific inbreeding  
143 estimated from runs of homozygosity can explain significant differences in hihi fitness, and if  
144 sex, age or lifespan predict individual reproductive success and survival.

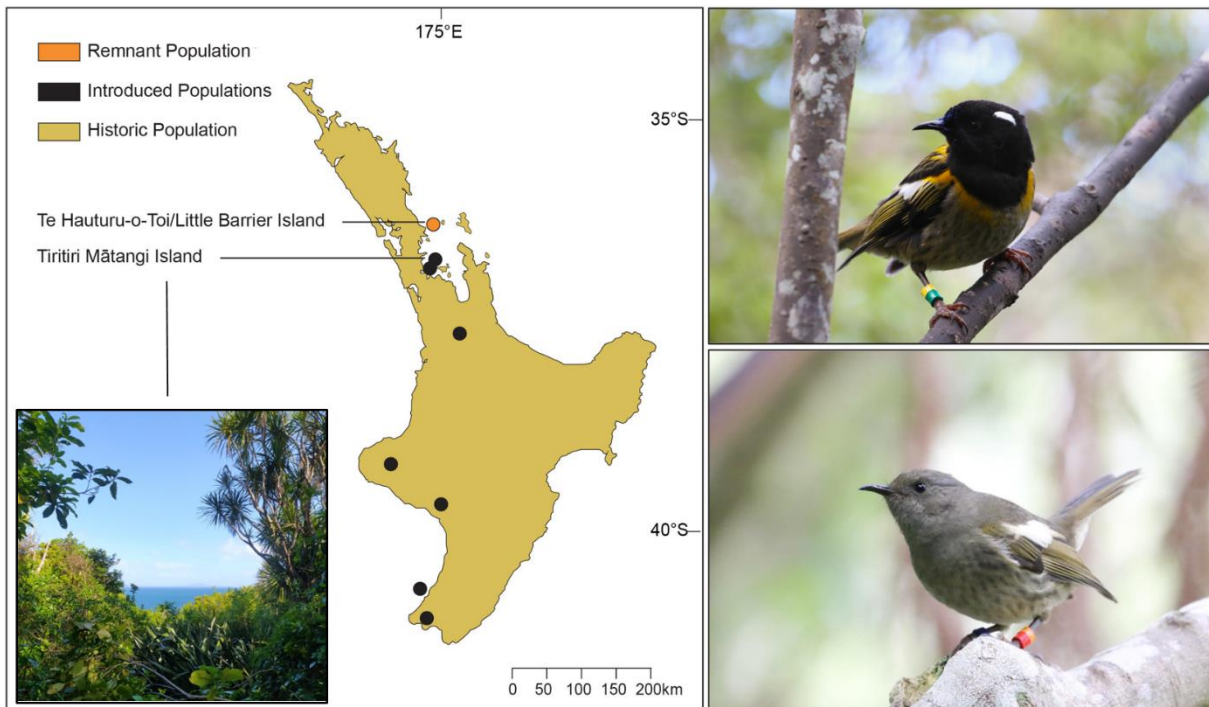
145 As far as we know, this is one of the first studies to report genome-wide and region-specific  
146 inbreeding based on extensive ROH metrics to investigate individual and temporal variation  
147 in inbreeding and to expose the effects thereof on fitness traits outside the field of animal  
148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

## 149 Materials and methods

### 150 Hihi population monitoring and sampling

151 Once abundant across the North Island, the endemic threatened hihi of Aotearoa New  
152 Zealand can now only be found in a single remnant island population on Te Hauturu-o-Toi  
153 (36°12'S, 175°05'E; Figure 1) and seven additional reintroduced populations in pest-free  
154 sanctuaries. Tiritiri Mātangi island is the largest of the latter populations, with a current  
155 population size of 192 hihi (Parlato et al., 2021). Hihi were first translocated from Te Hauturu-  
156 o-Toi to Tiritiri Mātangi in 1995 and since then have been subject to long-term study that  
157 includes the microsatellite genotyping of all individuals to establish complete pedigrees and  
158 the collection of extensive life history data. All fledglings are banded before they leave the  
159 nest, survival surveyed twice a year, and all reproductive attempts across the island

160 monitored over an individual's lifetime (Low & Part, 2009). This study originally included  
161 confirmed genotype data for 480 hihi from Tiritiri Mātangi (Duntsch et al., 2022). However,  
162 although there is no natural migration between hihi sites, 98 of the initial sample pool were  
163 translocated from Tiritiri Mātangi to other sanctuaries throughout their lives. To reduce  
164 population sub-structure in the dataset, a further 19 birds were excluded either because they  
165 had been translocated from Te Hauturu-o-Toi to Tiritiri Mātangi in 2010 or were offspring of a  
166 translocated bird. Hence, the final dataset for this project investigates the remaining 363  
167 individuals, which were sampled between 2001 and 2015, and for which complete survey  
168 and breeding data is available, as all sampled birds had died prior to the start of this study.



169  
170 **Figure 1: A map of the North Island of Aotearoa New Zealand showing the location of the only**  
171 **remnant hihi population on Te Hauturu-o-Toi and of the study population on Tiritiri Mātangi**  
172 **(shown in bottom left photo; taken by Laura Duntsch). Top right corner: a banded male hihi,**  
173 **taken by Stuart Attwood in Zealandia Sanctuary (the southern-most introduced population) and**  
174 **included with permission. Bottom right corner: a banded female hihi, taken by Melissa Boardman**  
175 **in Zealandia and included with permission.**

## 176 Genotyping and data filtering

177 In 2016, 1,536 hihi from five different populations were genotyped using a custom 50k  
178 Affymetrix single nucleotide polymorphism (SNP) array (Lee et al., 2022). This array was  
179 developed based on the identification of SNPs from de novo assembly of restriction site-  
180 associated DNA sequencing (RAD-seq) of 31 individuals from the Te Hauturu-o-Toi and  
181 Tiritiri Mātangi populations, and low-coverage whole-genome sequencing of ten of these  
182 birds (three of which were from Tiritiri Mātangi). SNPs were selected for approximately even  
183 genome spacing by mapping SNP positions from the highly fragmented assemblies to the  
184 zebra finch genome. Of the 58,466 SNPs included on the array, 45,553 markers passed  
185 initial quality control metrics in the Axiom Analysis Suite software (polymorphic SNPs with  
186 call rate of >95% and well-separated genotype clusters) and *PLINK* (Purcell et al., 2007)

187 filtering for minor allele frequency and Hardy-Weinberg equilibrium (--maf 0.01, --hwe 1e-20;  
188 Duntsch et al., 2021; Lee et al., 2022). The average genotyping rate across all samples is  
189 99.7%, with the mean per-site and per-individual missingness (--lmiss and --imiss in *PLINK*)  
190 not exceeding 0.26%. In 2021, those SNPs were mapped to contigs for a draft hihi reference  
191 genome (version NotCin10.4) and those genome contigs were then scaffolded into  
192 chromosomes based on homology to zebra finch and the hihi linkage map (*manuscript in*  
193 *preparation*). With sex chromosome positions removed, this resulted in a final dataset of  
194 41,195 SNPs with high-confidence assignment to 31 chromosomes. The SNP-containing  
195 contigs, scaffolded into chromosomes, cover 86% of the total estimated hihi genome size of  
196 1.06Gb (*manuscript in preparation*).

## 197 ROH-based inbreeding in hihi

198 Inbreeding was measured from the SNP data using the hidden Markov model-based  
199 approach in the R (R Core Team, 2013) package *RZooRoH* (Bertrand et al., 2019) that  
200 identifies homozygous-by-decent (HBD) segments (Druet & Gautier, 2017) and allows for the  
201 estimation of a global inbreeding coefficient. *RZooRoH* differs from some other inferences of  
202 ROH by providing a quantitative probability of a SNP being within an ROH, as well as  
203 reporting binary presence/absence, and therefore better captures uncertainty in ROH  
204 inference (Druet & Gautier, 2017; Bertrand et al., 2019). In a previous hihi study, we  
205 evaluated several models for the number of age-related HBD classes (K-1; the final class  
206 represents non-HBD) by varying K from 4 to 13, while allowing for a genotyping error rate of  
207 0.25% (Ferenčaković et al., 2013) and including a new option that improves the partitioning  
208 at higher inbreeding levels (layers=TRUE). The 13-class model was determined to be the  
209 best fit for hihi (Tom Druet, *personal communication*; Duntsch et al., 2021). Hence, the  
210 *RZooRoH* models for this study were fitted with the same rates as our previous study (of 10,  
211 20, 30, 40, 50, 100, 200, 500, 600, 700, 1000, 2000, 2000, where the final 2000 is the non-  
212 HBD class). When divided by two, these rates give an approximation of generation time  
213 since the most recent common ancestor for the segment falling into this HBD class (Bertrand  
214 et al., 2019). For each individual, HBD probabilities were summed over the first ten HBD  
215 classes (excluding HBD classes eleven (rate 1000) and twelve (rate 2000) that did not yield  
216 any HBD probabilities for the selected individuals) to give individual inbreeding coefficients  
217 ( $F_{\text{ROH}}$ ) for all birds.

218 We further divided the whole-genome inbreeding level into very recent, middle and ancient  
219 inbreeding ( $F_{\text{rec}}$ ,  $F_{\text{mid}}$ ,  $F_{\text{anc}}$ ). Very recent inbreeding  $F_{\text{rec}}$  includes HBD class 1 (up to  $10/2 = 5$   
220 generations), middle inbreeding  $F_{\text{mid}}$  includes the sum across HBD classes 2-6 (between 5  
221 and 50 generations) and ancient inbreeding  $F_{\text{anc}}$  includes the sum across HBD classes 7-10  
222 (between 50 and 350 generations). In more detail,  $F_{\text{rec}}$  includes homozygous segments that  
223 originated from inbreeding that happened since the 1990s, or since 5 generations ago, given  
224 the generation time of hihi of approximately 4.2 years as calculated from the pedigree. This  
225 interval therefore includes the year of the first two hihi translocations of 51 birds from Te  
226 Hauturu-o-Toi to Tiritiri Mātangi (in 1995 (38 birds) and 1996 (13 birds); of these 51, only 21  
227 survived to breed; Armstrong et al., 2002) and hence very recent and significant bottleneck  
228 events (Brekke et al., 2011). In addition, this first time point was chosen as it is most likely  
229 recent inbreeding events that have an effect on fitness (Makanjuola et al., 2020). Secondly,  
230 middle inbreeding  $F_{\text{mid}}$  was defined as the fraction of the whole-genome inbreeding level that

231 captures hihi dynamics after European arrival in Aotearoa New Zealand. More precisely, the  
232  $F_{mid}$  interval incorporates inbreeding levels accumulated between the time of the first  
233 European settlement in Aotearoa in 1822 and the start of the re-introductions of hihi to other  
234 islands and the mainland. Hihi were last seen on the mainland in 1883, and after cats and  
235 rats had led to a demise of hihi in the only remnant population on Te Hauturu-o-Toi, the  
236 island was declared a predator-free sanctuary in 1980 (Rasch et al., 1996). Lastly,  $F_{anc}$   
237 reflects hihi inbreeding more than 50 generations (~200 years) ago and hence is associated  
238 with the time before Europeans settled on the North Island, including Māori arrival (~1250,  
239 Supplementary Figure S1). Finally, we identified birds with very high and very low global  
240 inbreeding  $F_{ROH}$  and investigated the contribution of each of the HBD classes to their overall  
241 inbreeding coefficient, and their ROH density on a chromosome level.

242 We note that a probability-based *RZooRoH* approach will, on average, yield higher  
243 inbreeding values than binary estimates that are offered e.g. by *PLINK* (Meyermans et al.,  
244 2020), but our previous work indicates that these values are highly correlated for hihi  
245 (Duntsch et al., 2021; also confirmed herein for this set of birds by calculating genome wide  
246 inbreeding from *PLINK*, Supplementary Figure S2). As there is considerable variation in  
247 recombination rates across the macrochromosomes start-to-end, as well as sex differences  
248 in recombination rate for hihi (average recombination is 2.56 cM/Mb, *manuscript in*  
249 *preparation*), we present results in Mb using default *RZooRoH* functions and plots. The terms  
250 HBD and ROH are used interchangeably, and always refer to aspects of the *RZooRoH*  
251 output.

## 252 ROH density across the population

253 We measured the average ROH density across the genome for all Tiritiri Mātangi 363 hihi  
254 individuals by extracting all HBD segments per chromosome in *RZooRoH* and estimating  
255 mean HBD probabilities of all markers in non-overlapping 500kb windows using the R  
256 package *windowscanr* (Tavares, 2021), following R code provided by Stoffel et al. (2021a). In  
257 our dataset, the average marker density is 20 SNPs per 500kb window.

## 258 Modelling inbreeding depression, age and sex effects

### 259 Important hihi fitness traits

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

### 273 *MCMCglmm* modelling



274 The effects of whole-genome genomic inbreeding ( $F_{ROH}$ ), sex and lifespan on lifetime  
275 reproductive success (LRS), the effects of inbreeding, age and sex on annual reproductive  
276 success (ARS) and the effects of genomic inbreeding and sex on juvenile and adult annual  
277 survival (JUS, ADS) were tested using *MCMCglmm* (Hadfield, 2010), an R package that fits  
278 generalised linear mixed models using Markov chain Monte Carlo techniques. For LRS, fixed  
279 predictors included sex (male/female), lifespan (in years) and whole-genome inbreeding  $F_{ROH}$   
280 and the interaction between sex and  $F_{ROH}$ , while random effects included the breeding  
281 season in which bird was born (birth cohort) and the mother. LRS included lifespan to  
282 account for year-to-year stochasticity in survival rates and after confirming no significant  
283 impact of inbreeding on lifespan (see Supplementary Table S2c). LRS was fitted with a Zero  
284 inflated Poisson error distribution following de Villemereuil et al. (2019). For ARS, fixed  
285 effects of sex,  $F_{ROH}$  and age<sup>2</sup> (to reflect observed senescence in reproductive success) and  
286 their interactions were fitted, along with random effects of birth cohort, mother, ID and year of  
287 measurement. ARS was fitted with a Poisson error distribution. Finally, both annual adult  
288 survival (ADS) and juvenile survival (JUS) were fitted with interacting fixed effects of  $F_{ROH}$   
289 and sex, random effects of birth cohort, mother and ID, and a binomial error distribution  
290 (“threshold” family in *MCMCglmm*). Further, the ADS model random effects also included the  
291 year. Fixed and random components for LRS were included based on previous model  
292 selection for a larger LRS dataset (de Villemereuil et al, 2019) and were slightly modified for  
293 the other traits based on our biological understanding of the species.

294 We also fitted additional models with the separated inbreeding values ( $F_{rec}$ ,  $F_{mid}$ ,  $F_{anc}$ ), as  
295 very recent and middle inbreeding ( $F_{rec}$  and  $F_{mid}$ ) seemed to contribute most to inbreeding  
296 levels in highly inbred birds. All Bayesian models were run for 500,000 iterations after a burn-  
297 in period of 3,000, sampling every 20th output from the chain, a setting that resulted in a high  
298 minimal effective sample size for almost all fixed (>20,000) and random (>5,000) effects.  
299 Convergence was checked graphically and with the Heidelberger and Welch convergence  
300 test using the coda R package (Heidelberger & Welch, 1981; Plummer et al., 2006). We also  
301 fit all models above without interaction terms, and models of additive versus interaction fixed  
302 effects were compared with the deviance information criterion (DIC).

### 303 ROH genome wide association scan (GWAS)

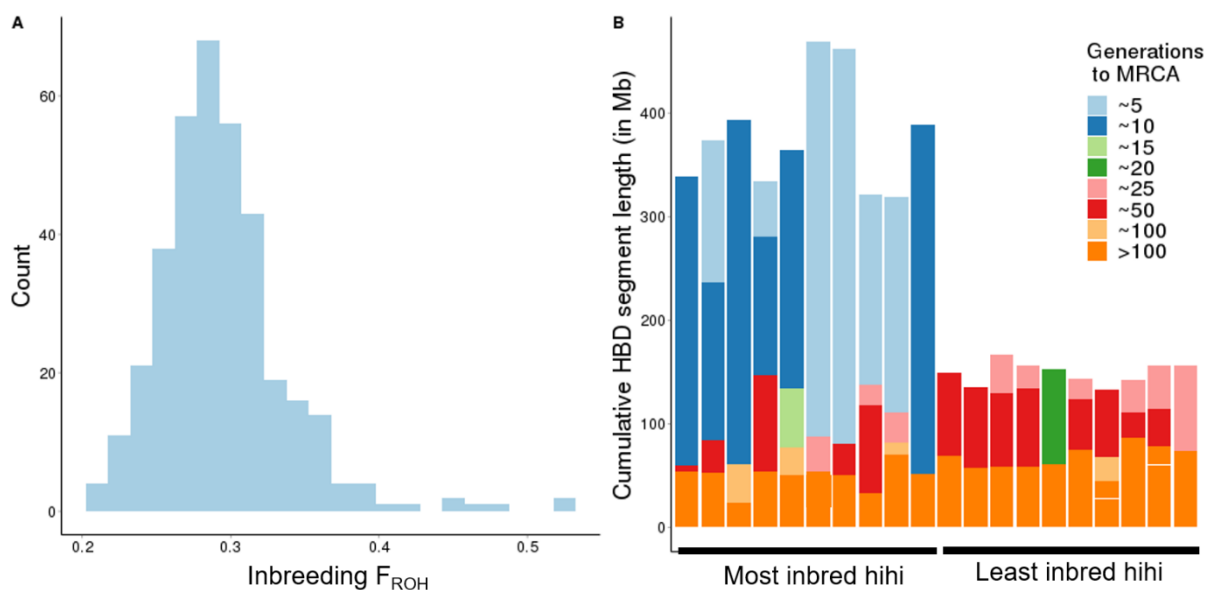
304 We also used the list of identified HBD-segments larger than 300kb from the *RZooRoH*  
305 analysis to test for association between an allele of a SNP being in a ROH and hihi fitness.  
306 *RZooRoH* categorises regions as being HBD when the HBD probability is >0.99 (Bertrand et  
307 al., 2019). Following the framework of Stoffel et al. (2021a), for each SNP we fitted a mixed  
308 model of association with fixed effects for each of the two SNP alleles and whether they were  
309 homozygous and in a ROH or not. The resulting p-values for the two predictors at each locus  
310 indicate whether a SNP in a ROH for specific allele is significantly associated with the trait,  
311 i.e., lifetime reproductive success, annual reproductive success, juvenile survival or adult  
312 survival. In addition to the two SNP allele effects, the top seven principal components of the  
313 variance-standardised additive relationship matrix (PC1–7; see Stoffel et al., 2021a) were  
314 fitted as fixed effects, in lieu of an additive genetic effect (although we note that these  
315 additive genetic effects are very small for hihi, see de Villemereuil et al., 2019). The  
316 remaining fixed and random predictors were included as selected from the best models from  
317 the above *MCMCglmm* analysis. Mixed models were fitted with the glmer function from the

318 *lme4* package (Bates et al., 2014). Both LRS and ARS glmer models were run with a  
 319 Poisson error distribution and the ADS and JUS models were run using the binomial  
 320 distribution (de Villemereuil et al., 2019). The conservative Bonferroni corrected threshold for  
 321 genome-wide significance was calculated using the common significance value  $p$  of 0.05 in  
 322 concordance with our previous GWAS analyses (Duntsch et al., 2020). All R scripts and  
 323 models regarding this mapping of inbreeding depression are modified from Stoffel et al.  
 324 (2021a) unless otherwise indicated, and can be found in the data availability section of their  
 325 publication together with a detailed description of their methods.

## 326 Results

### 327 ROH-based inbreeding in hihi

328 Inbreeding was measured using the R package *RZooRoH* that identifies homozygous-by-  
 329 decent segments (HBD; ROH). The probabilities to belong to each of the HBD classes were  
 330 summed across the genome to estimate the global genomic inbreeding coefficient. We found  
 331 that hihi on Tiritiri Mātangi have relatively high average inbreeding levels (mean  $F_{ROH} \sim 0.29$ ,  
 332 Figure 2A; Supplementary Figure S1 and Table S1). In total, we identified 89,061 HBD  
 333 segments across all 363 hihi (approximately 245 per individual), with the majority of  
 334 segments smaller than 300kb and in higher HBD classes (Supplementary Figure S3). The  
 335 largest HBD segment was more than 59 Mb long, the average length per segment was 0.9  
 336 Mb and the mean number of SNPs per identified HBD segment was 44. When only including  
 337 segments longer than 300kb, the average segment size is 1.4 Mb. Although higher on  
 338 average, the global total genomic inbreeding from *RZooRoH* was strongly correlated with the  
 339 measure from *PLINK* (*PLINK* average = 0.24, correlation = 0.99; Supplementary Figure S2).  
 340 Middle inbreeding (between 5 and 50 generations) shows the highest positive correlation  
 341 with total inbreeding, while ancient inbreeding was weakly negatively correlated with global  
 342 inbreeding (Supplementary Figure S4).



343

344 **Figure 2: A) Distribution of global (whole-genome) genomic inbreeding values  $F_{ROH}$  in the 363**  
 345 **Tiritiri Mātangi individuals as calculated with *RZooRoH*. B) The ten most inbred hihi (first set of**

346 bars) and the ten least inbred hihi (second set of bars) and the contribution of inbreeding over  
347 different timescales to the most recent common ancestor (MRCA) that make up their whole-  
348 genome inbreeding coefficient. Very inbred birds have high  $F_{ROH}$  because of very recent and  
349 recent inbreeding (light and dark blue bars, corresponding to HBD classes 1 and 2), indicating  
350 inbreeding within the last ten generations.

351  
352 In all highly inbred birds we find that most markers, when homozygous, have a high  
353 probability to be in a homozygous segment in the first two HBD classes (blue bars in Figure  
354 2B). This suggests the most recent common ancestors of these highly inbred birds have lived  
355 within the last 10 generations. All individuals show similar contributions to inbreeding from  
356 generations further in the past (dark orange bars in Figure 2B). In addition, birds with higher  
357 global inbreeding  $F_{ROH}$  also display more and longer HBD segments on the chromosome-  
358 level (Supplementary Figure S5).

### 359 ROH density across the population

360 For the population as a whole, we calculated the mean HBD probability in non-overlapping  
361 500kb windows across the genome. The mean window HBD probability never exceeded  
362 50%, suggesting high variation in regional inbreeding between individuals (Supplementary  
363 Figure S6). This means that no large stretches of the genome are strongly affected by  
364 inbreeding in all individuals, nor are there many genomic regions that do not have some  
365 degree of homozygosity.

### 366 Population averages of lifespan and reproduction

367 The average lifespan for the hihi presented in this study is 3.16 years (females: 2.9 years;  
368 males: 3.3 years) across all 363 birds, but 4.4 years (females: 4.2 years; males: 4.5 years)  
369 when only including birds that survive past their first year. The average total viable offspring  
370 number per individual equals 1.85 chicks (females: 2.0; males: 1.8) across all 363 birds but  
371 increases to 3.9 chicks when only taking breeding individuals into account. In our sample  
372 pool, there is no significant difference between males and females for lifespan (Welch Two  
373 Sample t-test, p-value = 0.1448) or total offspring numbers (Welch Two Sample t-test, p-  
374 value = 0.4634). In contrast, a one-way ANOVA revealed significant differences between  
375 birth cohorts for lifespan (p-value =  $7.49 \times 10^{-9}$ ; effect size 0.161) and total offspring numbers  
376 (p-value =  $2 \times 10^{-16}$ , effect size 0.264), with more recent hihi cohorts producing less offspring  
377 and having lower lifespans.

## 378 Inbreeding depression

### 379 Inbreeding effects on reproductive success and hihi survival

380 When testing for inbreeding depression in hihi from Tiritiri Mātangi island, we found that  
381 individual whole-genome inbreeding  $F_{ROH}$  significantly negatively affects juvenile survival  
382 (JUS;  $p=0.032$ ), but, although also estimated to have a negative effect, does not significantly  
383 affect annual reproductive success (ARS;  $p = 0.355$ ), adult survival (ADS;  $p = 0.534$ ) or  
384 lifetime reproductive success (LRS;  $p = 0.404$ ; Table 1; Supplementary Tables S2a, S3, S4)  
385 of an individual. Moreover, juvenile survival is significantly affected by the sex of an  
386 individual, with males more likely to survive than females ( $p = 0.029$ , Supplementary Figure  
387 S7). The interaction term of sex with inbreeding is also significant ( $p = 0.02$ ), suggesting that  
388 more inbred females are less likely to live past the juvenile stage than less inbred ones,  
389 whereas male juvenile survival is not as affected by inbreeding. Further, we found hihi

390 lifespan to be the only predictor significantly associated with lifetime reproductive success  
 391 (LRS) of an individual hihi, with older birds having higher total offspring numbers than birds  
 392 that die young ( $p = 0.000$ ). In our models, only the age of an individual significantly predicts  
 393 its annual reproductive success (ARS), with both males and females having most of their  
 394 successful offspring between the ages of 3-7 ( $p=0.000$ ; Supplementary Figure S7). Finally,  
 395 we detected no significant association of inbreeding or sex with our last fitness component,  
 396 adult annual survival (ADS; Table 1, Supplementary Table S4).

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

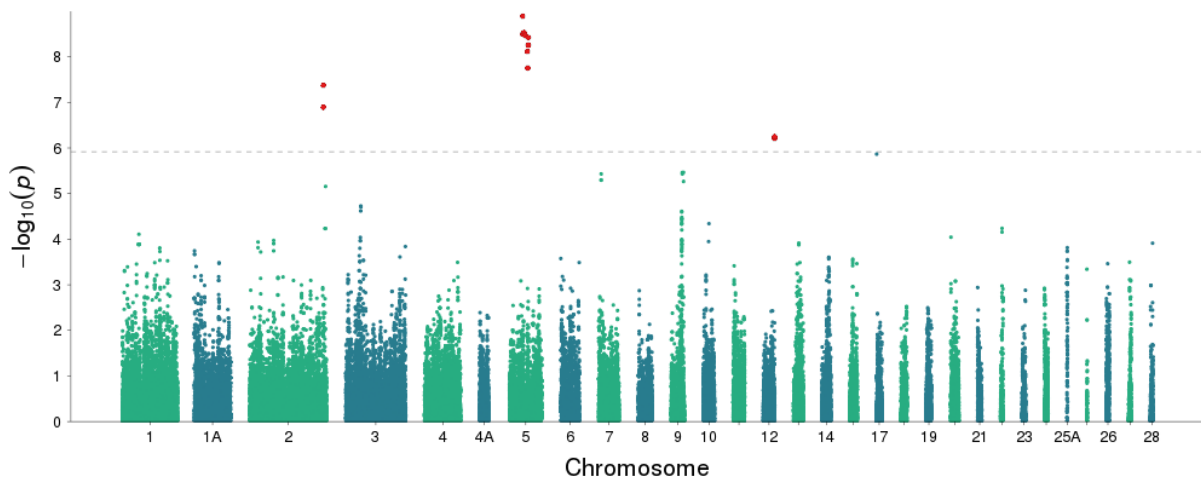
<b>MCMCglmm</b>	<b>Predictor</b>	<b>Post.mean</b>	<b>Lower 95% CI</b>	<b>Upper 95% CI</b>	<b>pMCMC</b>
LRS ~ $F_{ROH}$ * Sex + Lifespan	$F_{ROH}$	-2.395	-8.101	3.190	0.404
	Sex	-0.107	-2.132	1.916	0.919
	Lifespan	0.387	0.327	0.450	<b>0.000</b>
	$F_{ROH}$ :Sex	-0.598	-7.469	6.389	0.861
ARS ~ $F_{ROH}$ * Sex + Age <sup>2</sup>	$F_{ROH}$	-2.554	-7.899	2.949	0.355
	Sex	0.527	-1.400	2.385	0.578
	Age	0.376	0.320	0.434	<b>0.000</b>
	$F_{ROH}$ :Sex	-2.980	-9.552	3.426	0.369
JUS ~ $F_{ROH}$ * Sex	$F_{ROH}$	-6.964	-13.375	-0.344	<b>0.032</b>
	Sex	-2.466	-4.780	-0.245	<b>0.029</b>
	$F_{ROH}$ :Sex	8.786	0.949	16.157	<b>0.021</b>
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

405 We further partitioned global inbreeding into very recent inbreeding since their first  
 406 translocation to the island ( $F_{rec}$ ; since 1990), middle inbreeding since European arrival to  
 407 Aotearoa New Zealand ( $F_{mid}$ ; since 1822) and ancient inbreeding ( $F_{anc}$ ; Supplementary  
 408 Figure S1), and tested the effects thereof on all four fitness traits. Firstly, none of the  
 409 separated inbreeding values have a significant negative effect on lifetime reproductive  
 410 success (LRS; Supplementary Table S6), even though the two inbreeding measures of  
 411 middle inbreeding  $F_{mid}$  and ancient inbreeding  $F_{anc}$  show a weak negative correlation with  
 412 LRS (Supplementary Figure S8). Similarly, annual reproductive success (ARS) is unaffected  
 413 by the three partitioned inbreeding measures. Lastly, none of the partitioned inbreeding  
 414 measures predicted hihi juvenile (JUS) or adult annual survival (ADS; Supplementary Table  
 415 S6).  
 416

## 417 GWAS

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

423 Even though the *MCMCglmm* analysis revealed no significant effect of global inbreeding on  
424 our main fitness trait, our genome wide association for inbreeding revealed that the ROH  
425 status of thirteen SNPs was significantly negatively associated with lifetime reproductive  
426 success (LRS; Figure 3; Supplementary Figure S9). These SNPs cluster on three different  
427 chromosomes, chromosome 2 (two SNPs), chromosome 5 (nine SNPs) and chromosome 12  
428 (two SNPs), and have similarly large effect sizes (Supplementary Table S7). In contrast,  
429 none of the SNPs when homozygous and in a ROH was significantly associated with annual  
430 reproductive success (ARS), in agreement with the lack of effect of whole-genome  
431 inbreeding on that trait (Supplementary Figure S10). Similarly, we did not detect any  
432 significant association of the ROH status of SNPs with juvenile or adult annual survival, with  
433 all p-value estimates well below the conservative Bonferroni significance threshold  
434 (Supplementary Figure S10, qq-plots in Supplementary Figure S11).



435 **Figure 3: Manhattan plot showing that thirteen of the SNPs, when homozygous and in a ROH,**  
436 **tested in our interaction model have a significant negative effect on fitness (LRS) in the**  
437 **population of hihi on Tiritiri Mātangi. The dotted line is the conservative Bonferroni corrected**  
438 **threshold.**  
439

## 440 Discussion

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

## 448 Genome-wide and regional inbreeding

449 In this study, we find that genome-wide inbreeding levels for the 363 hihi from the Tiritiri  
450 Mātangi population are relatively high ( $F_{\text{ROH}}^{\text{RZooRoH}} = 0.29$ ,  $F_{\text{ROH}}^{\text{PLINK}} = 0.24$ ) when comparing  
451 to inbreeding levels in all remaining individuals of another threatened Aotearoa New Zealand  
452 bird species, the kākāpō (*Strigops habroptilus*;  $F_{\text{ROH}}^{\text{PLINK}} = 0.18$ ; using 12,241 SNPs; Foster  
453 et al., 2021). Hihi ROH-based inbreeding is also much higher than in a population of a US  
454 endemic avian species of conservation concern, the Florida scrub jay (*Aphelocoma*  
455 *coerulescens*;  $F_{\text{ROH}}^{\text{PLINK}} = 0.012$ ; using 11,737 SNPs; Nguyen et al., 2022), which suggests  
456 that expected inbreeding levels in threatened species will vary depending on extant and past  
457 population size, recent demographic history, life history, natural dispersal and management  
458 strategies (de Kort et al., 2021). Further, we were able to show that many of the hihi  
459 individuals with very high genome-wide inbreeding levels have experienced inbreeding  
460 recently, within the last 5 generations, which coincides with the timing of the original  
461 translocation of 21 surviving birds from Te Hauturu-o-Toi in the mid-nineties as well as  
462 subsequent reinforcements to Tiritiri Mātangi (Brekke et al., 2011; de Villemereuil et al.,  
463 2019). Many highly inbred individuals also display higher inbreeding levels due to events  
464 towards the middle of the last century (~10 generations ago), when hihi were long gone from  
465 the mainland. During that time, a drastic decline of hihi in the last remnant population on Te  
466 Hauturu-o-Toi due to rat and cat predation was recorded, presenting yet another bottleneck  
467 event that hihi underwent in the last century (Rasch et al., 1996). Together, our findings of  
468 enhanced inbreeding levels on Tiritiri Mātangi agree with previous studies detecting fewer  
469 polymorphic sites in the Tiritiri Mātangi population compared to the remnant Te Hauturu-o-  
470 Toi population, albeit these studies had small sample sizes (Brekke et al., 2011; de  
471 Villemereuil et al., 2019). In addition, the ROH analysis revealed that, although the majority  
472 of runs of homozygosity are small, some regions of homozygosity are very large, spanning  
473 up to 59Mb (60% of chromosome 1). This is much longer than, for example, the longest run  
474 of homozygosity (17.5 Mb) in a large-scale collared flycatcher (*Ficedula albicollis*) study  
475 using 104 re-sequenced genomes (Kardos et al., 2017), while more comparable to highly  
476 inbred kākāpō displaying ROH sizes of up to 75.61Mb (Foster et al., 2021). However, on a  
477 population scale, we detected notable variation in inbreeding and inbreeding patterns  
478 between individuals, and no genomic region was inbred in more than 50% of individuals.  
479 Similar findings were reported in a farmed rainbow trout population, with high variation in  
480 individual inbreeding and chromosomal inbreeding levels along the genome (Paul et al.,  
481 2021).

## 482 The fitness consequences of inbreeding

483 Given the mostly low effective population sizes, close relationships and variable selection  
484 pressures in species of conservation concern (Ceballos et al., 2018), understanding the  
485 direct fitness consequences of inbreeding is a topic of high priority for threatened species  
486 such as hihi. We know that the number of loci genotyped with the custom hihi SNP array are  
487 sufficient to detect runs-of-homozygosity in over 88% of the autosomal genome, and hence  
488 provide enough power to detect inbreeding-fitness correlations in this population (Miller &  
489 Coltman, 2014; Duntsch et al., 2021). When correlating whole-genome inbreeding levels with  
490 four important fitness traits for individuals from the Tiritiri Mātangi population, we were able to  
491 detect a significant negative effect of global inbreeding on hihi juvenile survival. Hence this  
492 study is a valuable addition to previous inbreeding depression studies investigating the

493 effects of inbreeding on traits related to reproduction and survival in small populations  
494 (Hansson & Westerberg, 2002; Keller & Waller, 2002; Billing et al., 2012; Hoffman et al.,  
495 2014b; Kennedy et al., 2014; Hoeck et al., 2015; Hedrick & Garcia-Dorado, 2016; Howard et  
496 al., 2017; de Boer et al., 2018; Kardos et al., 2018; Willoughby et al., 2019; Flanagan et al.,  
497 2021; Foote et al., 2021; Khan et al., 2021; Vega-Trejo et al., 2022).

498 Our study suggests that global inbreeding has a significant negative effect on hihi juvenile  
499 survival, where less inbred individuals are more likely to survive past the juvenile stage than  
500 highly inbred birds. Furthermore, the effect of inbreeding on juvenile survival appears to differ  
501 between the sexes, with inbred females showing higher mortality within their first year than  
502 inbred males. In the past, inbreeding has been shown to have greater impact on male  
503 compared to female hihi survival at early stages of development based on microsatellite  
504 markers (Brekke et al., 2010). Further, a male bias in mortality has been observed in the  
505 population at the embryo development and nestling stage (Fay Morland, *personal*  
506 *communication*), whereas our study suggests higher inbred female mortality later in their  
507 juvenile life. Therefore, inbreeding may impact hihi at slightly different stages of development  
508 between males and females. Overall, this implies that highly inbred hihi individuals may be  
509 removed from the Tiritiri Mātangi population before they can reproduce, a scenario that  
510 would see a decrease of the overall levels of inbreeding in the breeding part of the  
511 population. In addition, we show that annual reproductive success is age-dependent in the  
512 hihi population, with birds younger than 3 years and older than 7 years showing lower annual  
513 offspring numbers, agreeing with previous findings of senescence in hihi (Low & Part, 2009).  
514 Our results are supported by various studies that investigated sex-specific inbreeding  
515 depression effects in wild populations (Billing et al., 2012), reporting faster reproductive  
516 senescence for inbred females but not for males and different relationships between  
517 inbreeding, age and disease susceptibility for males and females (Benton et al., 2018). In the  
518 future, it will remain important to consider factors such as sex and senescence when  
519 evaluating the genetic health of a population, as inbreeding depression across different life  
520 stages and sexes remains to be fully understood (Trask et al., 2021; Vega-Trejo et al., 2022).

521 The fact that we could not detect a significant effect of individual whole-genome inbreeding  
522 on lifetime reproductive success, annual reproductive success and annual adult survival  
523 suggests that genome-wide inbreeding may not fully capture the inbreeding load of  
524 individuals, there is considerable variation in the impacts of inbreeding over time, and/or we  
525 may lack power to detect a significant impact of inbreeding on these traits. While we detected  
526 a significant effect of inbreeding on some but not all chosen fitness proxies on Tiritiri Mātangi,  
527 this does not prove the absence of an inbreeding effect on other traits (Altman & Bland,  
528 1995), especially with regard to the large credible intervals. We also suspect that the  
529 population is experiencing weaker selection against deleterious variants and can tolerate  
530 higher levels of inbreeding as long as supplementary feeding is provided and environmental  
531 conditions are ideal (Armstrong et al., 2007; Chauvenet et al., 2012; Ewen et al., 2015). It is  
532 also worth noting that the population size of hihi on the island has increased steadily over the  
533 course of this study, with a total population size of 100 birds in 2004, to 170 individuals in  
534 2015, potentially increasing competition for territories, resources and mates, while reducing  
535 chances to mate with a close relative. However, we are aware that multiple factors can  
536 contribute to a lack of power to detect whole-genome inbreeding effects on our main fitness  
537 trait. While we included the most obvious predictors such as sex and age of the individual in

538 our mixed models, and fitted birth cohort, year, mother and ID as random effects, additional  
539 environmental effects that have been unaccounted for might in fact be the main drivers of  
540 fitness in adult birds. Those factors include but are not limited to fluctuations of the average  
541 temperature of each year, droughts and heavy rainfall, natural food availability and  
542 phenology, and microclimate effects and hihi density per individual territory. Moreover, the  
543 most important contributors to fitness may vary depending on the population across the  
544 North Island (Rutschmann et al., 2020). Other hihi populations, such as Zealandia sanctuary,  
545 Wellington, are smaller, surrounded by different types of flora and co-exist with different  
546 avian species than Tiritiri Mātangi, hence could be affected differently by inbreeding. Future  
547 studies may also measure genomic inbreeding depression for additional components and  
548 key adaptive traits such as body size, mating success, breeding strategy, traits measured at  
549 different development stages or annual fitness in the first year of life (Alif et al., 2022).

### 550 **Genome-wide association of inbreeding depression**

551 High variation in inbreeding landscapes between individuals might mask the effect of regional  
552 inbreeding on important hihi fitness traits when the overall genome-wide inbreeding effect is  
553 non-significant (Slate et al., 2004; Paul et al., 2021). We conducted a genome-wide  
554 association study for inbreeding depression by using the ROH status of a SNP as a predictor  
555 of fitness, in order to further investigate inbreeding effects on our four measured fitness  
556 components. This regional genomic approach was able to detect thirteen loci with negative  
557 effect on lifetime reproductive success of hihi, a finding that would have gone unnoticed  
558 using a genome-wide inbreeding approach alone. These SNPs are located on chromosome  
559 2 (two SNPs mapping to positions 137,385,541 and 137,597,845), a large region across  
560 chromosome 5 (nine SNPs mapping to positions 25,343,922 to 35,850,425) and  
561 chromosome 12 (two SNPs mapping to position 21,012,542 and 21,112,406). Some of these  
562 loci are located near (within a few hundred kb upstream and downstream) of protein-coding  
563 genes, according to the zebra finch genome annotation (assembly version number  
564 bTaeGut1\_v1.p). Examples are *EHD4* (enables cadherin binding activity) and *SMOC1* (a  
565 Calcium-Binding Protein) on chromosome 5 and *EXT1* (Exostosin Glycosyltransferase) on  
566 chromosome 2, a putative tumour suppression protein. All of the SNPs with negative effect  
567 on fitness represent minor alleles, with their allele frequency ranging from 0.06 to 0.24 (see  
568 Supplementary Table S7), which suggests that these SNPs may be in linkage disequilibrium  
569 with recessive deleterious mutations generating inbreeding depression by appearing in a  
570 homozygous state in inbred individuals. It is important to note that some important genomic  
571 regions may not be well-tagged by the genotyped SNPs, e.g., no SNPs were successfully  
572 genotyped on micro-chromosome 16, which is thought to contain the major histocompatibility  
573 complex (MHC; Lee et al., 2022).

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



582 for the three remaining fitness components in hihi.

### 583 [Hihi conservation genomics](#)

584 Hihi are extremely vulnerable to all predators and competitors as well as a change in climate.  
585 Hence, understanding the genetic architecture of crucial fitness traits in the main source  
586 population for translocations of hihi, Tiritiri Mātangi island, can help us understand just how  
587 compromised the species' evolutionary capacity is (de Villemereuil et al., 2019; Duntsch et  
588 al., 2020). We were able to detect effects of inbreeding depression in hihi on Tiritiri Matangi  
589 island, while previous studies have found reduced adaptive potential given a low additive  
590 genetic variance of fitness in the species (de Villemereuil et al., 2019; Bonnet et al., 2022).  
591 This potentially suggests that the small but non-zero genetic contribution to fitness  
592 differences was captured because of the presence of moderately deleterious variants, that  
593 have failed to be purged from the population, possibly because they were recessive rather  
594 than co-dominant or dominant). These moderate-effect deleterious recessive mutations may  
595 have increased in frequency over time due to drift (Hedrick & Garcia-Dorado, 2016) and the  
596 relatively high levels of inbreeding have exposed genetic load from these recessive  
597 mutations, leading to inbreeding depression. In support of this hypothesis, it is notable that  
598 we have detected significant association of the homozygous state of thirteen SNPs with  
599 fitness, which would not be expected if inbreeding depression was explained solely by many  
600 very small effect loci (albeit we acknowledge that the SNP effect sizes are likely  
601 overestimated; Slate et al., 2013). From a species conservation perspective, this indicates  
602 that hihi mean fitness is expected to further decrease if inbreeding rates increase in the Tiritiri  
603 Matangi population. Future conservation genomic work for hihi might therefore not focus only  
604 on the study of rare adaptive alleles, but more importantly on the detection and mitigation of  
605 the accumulation of recessive deleterious mutations in all translocated populations.

606 While the role of purging on survival and reproductive success has been discussed for many  
607 mammalian species with long-term pedigree data (Kyriazis et al., 2022), this kind of insight is  
608 still scarce in birds and threatened species in general. That being said, the exact status of  
609 hihi genomic vulnerability remains to be assessed by future whole-genome sequencing  
610 approaches, which will enable us to take a closer look at the loci and regions contributing to  
611 inbreeding depression in hihi discovered in these analyses, alongside an analysis of  
612 selection and diversity in the genome. Future simulations will also show whether inbreeding  
613 depression is likely to translate into reduced population growth and recovery (Johnson et al.,  
614 2011). Our results advocate for and support the move away from only testing the fitness  
615 effects of global whole-genome inbreeding values to also investigating the impacts of region-  
616 specific inbreeding estimates for a species of conservation concern, as global inbreeding  
617 alone might not be a suitable estimation of genetic health in all wild populations. This will  
618 more reliably capture the true inbreeding landscape of individuals in small populations and  
619 help to recognize, monitor and mitigate the fitness consequences of bottleneck events.

620

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949 ened Aotearoa New Zealand passerine.



## 950 Data Accessibility

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

## 958 Benefit Sharing

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

## 964 Author Contributions

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