

10 **Abstract**

11 To predict if a threatened species can adapt to changing selective pressures, it is crucial to
12 understand the genetic basis of adaptive traits, especially in species historically affected by
13 severe bottlenecks. We estimated the heritability of three hihi (*Notiomystis cincta*)
14 morphological traits known to be under selection: nestling tarsus length, body mass and head-
15 bill length, using 523 individuals and 39,699 single nucleotide polymorphisms (SNPs) from a
16 50K Affymetrix SNP chip. We then examined the genetic architecture of the traits via
17 chromosome partitioning analyses and genome-wide association scans (GWAS). Heritabilities
18 estimated using pedigree relatedness or genomic relatedness were low. For tarsus length, the
19 proportion of genetic variance explained by each chromosome was positively correlated with
20 its size, and more than one chromosome explained significant variation for body mass and
21 head-bill length. Finally, GWAS analyses suggested many loci of small effect contributing to
22 trait variation for all three traits, although one locus (a SNP within an intron of the transcription
23 factor *HEY2*) was tentatively associated with tarsus length. Our findings suggest a polygenic
24 nature for the morphological traits, with many small effect size loci contributing to the majority
25 of the variation, similar to results from many other wild populations. However, the small
26 effective population size, polygenic architecture and already low heritabilities suggest that both
27 the total response and rate of response to selection are likely to be limited in hihi.

28

29 *Keywords: genomic relatedness matrix; chromosome partitioning; genome-wide association;*
30 *polygenic traits; pedigree study; Notiomystis cincta*

31 **1 Introduction**

32 In small populations, genetic diversity is eroded over time through genetic drift, including
33 events such as population bottlenecks and founding events (Bouzat, 2010). The continual loss
34 of genetic diversity is of particular interest in species of conservation concern with already low
35 levels of variation. The erosion of genetic diversity may result in the loss of putatively adaptive
36 alleles, leaving the species with low adaptive potential when facing new selection pressures,
37 such as a change in climate or habitat fragmentation (Hoffmann et al., 2017, Chen et al., 2016,
38 de Villemereuil et al., 2019b). However, the complex genomic architecture of traits in non-
39 model organisms is often unknown and, consequently, the underlying evolutionary processes
40 and possible ecological consequences for a population or a species subject to selection cannot
41 be directly assessed (Savolainen et al., 2013). Recently, with the advent of modern and
42 inexpensive sequencing and genotyping technologies, the field of conservation genomics has
43 entered a new era where it is possible to genotype a large number of markers for threatened
44 species with small population sizes (Narum et al., 2013). Now, the challenge at hand is to
45 adequately analyse and interpret these genomic snapshots in a way that we can predict the
46 adaptive potential for threatened species and begin to inform conservation management
47 (Harrisson et al., 2014; Shafer et al., 2015).

48
49 Genomic approaches have already proven to be a powerful tool to better understand the
50 structure and evolution of adaptive traits (Pardo-Diaz et al., 2015; Luikart et al., 2018). For
51 example, a closer look at the genetic basis of horn polymorphism in Soay sheep (*Ovis aries*)
52 revealed that variation is maintained by heterozygote advantage at a single gene (Johnston et
53 al., 2013), which would be impossible to infer from pedigree-based methods alone. Hence,
54 today, many population genomic studies aim to investigate the genetic basis of variation in life-
55 history, fitness and morphological traits in their organism of interest (Luikart et al., 2018). To
56 make assumptions about adaptive potential, it is crucial to understand the proportion of the
57 phenotypic variation observed in a population (V_P) that is explained by the additive genetic
58 component (V_A), a factor referred to as narrow-sense heritability (h^2) (Falconer and Mackay
59 1996). Traditionally, h^2 has been estimated using the expected relatedness between
60 individuals captured by pedigree data. However, those pedigrees may contain errors, not
61 capture all relationships between sampled individuals due to shallow and inconsistent pedigree
62 depth, and only capture expected, rather than realised, genome sharing between two related
63 individuals (Visscher et al., 2006). Therefore, it has recently become common practice to
64 instead use genomic data when estimating trait heritabilities (Gienapp et al., 2017b), for
65 example via inferring the realised genome sharing between individuals described by a genomic

66 relatedness matrix (GRM). Assuming that marker density is sufficient to accurately capture
67 genomic relatedness, GRM-based heritabilities can be more precise, providing valuable insight
68 into the evolutionary potential of the species (Berenos et al., 2014, Gienapp et al., 2017b,
69 Jensen et al., 2014).

70 In addition to characterising the overall GRM-based heritability, the heritability can be
71 partitioned across regions of the genome to give further insight into the genomic basis of trait
72 variation (Yang et al., 2011b). If large chromosomes, containing more genes, explain a more
73 substantial proportion of the genetic variation compared to smaller chromosomes, the trait is
74 likely polygenic – i.e. there are many loci, scattered throughout the genome, contributing to
75 trait differences (Yang et al., 2011b, Robinson et al., 2013). More than one chromosome
76 explaining significant variation also suggests that many loci contribute to trait variation
77 (Kempainen & Husby, 2018a).

78 Another way of investigating the genetic basis of adaptive traits using genomic data involves
79 a scan for genome-wide associations (GWAS). This detects specific loci with small to large
80 effect on the phenotype of the study species. The power to detect association is dependent,
81 among other factors, on the effect size of the locus. If a trait with significant heritability has no
82 significant GWAS peaks, it suggests that the trait is polygenic, with many loci of small effect
83 contributing to variation (Manilo et al., 2009). A further hurdle to finding significant genome-
84 wide associations is presented when individuals are closely related, which is commonly the
85 case when sampling from natural populations, particularly those with small population size.
86 The similarity in genetic background must first be adjusted for before testing for an association,
87 which may further reduce the power to detect an association (Yang et al., 2014).

88

89 It appears that a polygenic architecture underlies many evolutionarily significant traits under
90 investigation (Jensen et al., 2014; Kardos et al., 2016). Variation in these traits is maintained
91 by the balance between genetic drift, mutation and selection, with smaller heritabilities
92 predicted in populations with small effective population size (Hoffman et al., 2017). For these
93 populations, theoretical and simulation results suggest that polygenic adaptation in response
94 to selection is likely to proceed via allele frequency changes at a subset of loci with large effect
95 and/or high allele frequency, rather than frequency shifts across all loci (Höllinger et al., 2019).
96 Variation at other loci is susceptible to be lost via genetic drift and will, therefore, constrain
97 both the rate of response and the total response to selection (John & Stephan, 2020). In
98 addition, the evolution of polygenic traits can be further constrained by linkage disequilibrium
99 between deleterious and beneficial alleles, and trade-offs with other (potentially unmeasured)
100 traits (Hill & Robertson 1966; Hospital & Chevalet 1996; Lande 1982). Therefore,

101 understanding the genetic architecture of adaptive traits is likely to enable better predictions of
102 the adaptive potential of small populations.

103

104 The hihi is an endemic passerine of Aotearoa | New Zealand and is becoming a model species
105 for studying the genetics of endangered birds, and species with small population sizes more
106 generally (Brekke et al., 2011; de Villemereuil et al., 2019a; de Villemereuil et al., 2019b). The
107 hihi dataset on the island of Tiritiri Matangi (36°36'S, 174°53'E) contains a multi-generational
108 pedigree and morphological and life history data for every individual born since 1995 when the
109 population was established through reintroduction. Recently, de Villemereuil et al. (2019a,
110 2019b) predicted little adaptive potential of this species based on signatures of low genetic
111 diversity, low additive genetic variance of fitness, and small pedigree-based heritabilities for
112 traits under selection. All but one life-history trait had very small heritabilities (<0.0001),
113 however, there was good support for low but non-zero heritabilities for beak length, body mass
114 and tarsus length (de Villemereuil et al., 2019b). While previous hihi studies already examined
115 the age-dependent effect of body size on fitness (Low 2006), this large dataset (>3000 birds)
116 for the first time revealed that all three morphological traits had positive linear selection and
117 negative non-linear selection gradients (de Villemereuil et al., 2019b). These gradients suggest
118 that there are optimal values for nestling tarsus length, head-bill length and mass that maximise
119 fitness, but that all trait means are significantly smaller than these optima, and hence are under
120 directional selection to increase body size. Morphological traits are commonly recorded in
121 avian studies, with body size directly associated with fitness in many species (reviewed in
122 Merilä and Sheldon, 2001; Gardner et al., 2011), and are the focus of a growing number of
123 genomic studies (reviewed in Husby et al., 2019).

124

125 Here, we used genomic data to reveal previously undiscovered genetic patterns underlying
126 these three adaptive morphological phenotypes in the Tiritiri Matangi population. We employed
127 state-of-the-art software and data for 523 individuals genotyped on a recently generated
128 custom SNP array to generate heritability estimates from pedigree relatedness and genomic
129 relatedness, testing whether a genomic snapshot provides comparable results to a pedigree
130 over many generations for the additive genetic variance component of the traits. To explore
131 the genomic architecture of each trait, we further partitioned the variance explained by the
132 genotyped SNPs across chromosomes to test for a possible polygenic architecture. Finally,
133 we performed a genome-wide association study in order to find loci of small, medium and large
134 effect on any of the traits under investigation. For traits with low heritability, we wanted to
135 discern whether a GWAS could detect loci of large effect, or whether there was evidence that

136 traits are controlled by many loci of small effect, in agreement with studies on many other wild
137 populations. From a conservation management perspective, a low heritability estimate and
138 polygenic makeup of these morphological traits will complement our previous work
139 demonstrating that hihi has little adaptive potential when facing future changes in selection
140 pressures.

141

142 **2 Methods**

143 **Study population and phenotypic data**

144 The hihi was once abundant on the North Island of Aotearoa, until habitat loss and predation
145 by introduced mammals confined its range to only one naturally occurring population on Te
146 Hauturu-o-Toi (Little Barrier Island), in the Hauraki Gulf (36°12'S, 175°05'E) (Brekke et al.,
147 2011). In 1995, the hihi was successfully introduced to the nearby island of Tiritiri Matangi, with
148 a population size of 210 birds prior to the 2019/2020 breeding season (Parlato et al., *in review*).
149 As a result of extensive monitoring since its translocation, we now have a wealth of
150 environmental, life history, fitness, morphological and pedigree data for the population (de
151 Villemereuil et al., 2019b). Hihi do not migrate between the populations and predominantly
152 nest in nest boxes on Tiritiri Matangi Island. During every breeding season (ranging from
153 October through to February), nestlings are banded and measured for body mass (g), but also
154 for tarsus length (mm) and head-to-bill length (mm) as those two measures stabilize after 21
155 days. Blood has been routinely taken (around 60µl per individual via brachial vein puncture)
156 from nestlings since 2004.

157

158 **Genotyping and pedigree data**

159 Due to a very high rate of extra-pair copulations in this species, the paternity is determined via
160 routine genotyping of a panel of 19 microsatellite markers, allowing the construction of a multi-
161 generational pedigree for the population (for details see Brekke et al., 2012; de Villemereuil et
162 al., 2019a). For nestlings without blood samples available (i.e. those born before 2004), fathers
163 are assumed to be unknown in the pedigree. In addition, in 2016, 1,536 birds (from five different
164 hihi populations) were genotyped using a custom 50k Affymetrix single nucleotide
165 polymorphism (SNP) array (manuscript *in prep*). This array was developed based on the
166 identification of SNPs from *de-novo* assembly of restriction site-associated DNA sequencing
167 (RAD-seq) of 31 individuals from the Te Hauturu-o-Toi and Tiritiri Matangi populations, and
168 low-coverage whole-genome sequencing of ten of these birds (three of which were from Tiritiri
169 Matangi). The flanking sequences of identified variants were aligned to the Ensemble 86 zebra
170 finch (*Taeniopygia guttata*) genome, which, given the very high synteny of avian genomes

171 (Zhang et al., 2014), is used in the following analyses as a proxy for the distribution of markers
172 in the hihi genome (electronic supplementary material, table S1).

173

174 Of the 58,466 SNPs included on the array, 45,553 markers were designated as polymorphic
175 high resolution according to the default quality control metrics in the Axiom Analysis Suite
176 software. *PLINK* (Purcell et al., 2007) filtering (--maf 0.01, --hwe 1e-20) resulted in the removal
177 of 325 and 69 loci among the autosomes, respectively. 2,734 SNPs appeared as duplicates
178 (i.e. mapped to the same chromosome at the same position) and hence only one SNP from
179 each position was retained. To avoid population structure as a confounding factor, we only
180 included birds from Tiritiri Matangi Island in this study, which shows no structure (electronic
181 supplementary material, figure S1). No individual was removed due to low genotyping rates (-
182 -mind), but some were excluded for having incomplete phenotypic information. The final
183 dataset contained 39,699 autosomal genomic loci with a mean call rate of 0.997398 and
184 complete sex and phenotype information on all three traits for 523 birds.

185

186 **Trait heritabilities**

187 We employed several different methods of retrieving heritability estimates for our three traits
188 under investigation, using generalised linear mixed models (an ‘animal model’, Wilson et al.,
189 2009) and either the pedigree relatedness or the relatedness estimated from the genotyped
190 markers (i.e. the genomic relatedness matrix; GRM). First, pedigree- and GRM-based
191 heritabilities (h^2_{ped} , h^2_{GRM1}) were estimated in a Bayesian framework using the R (R Core Team,
192 2013c) package *MCMCglmm* (Hadfield 2010). To estimate h^2_{ped} , we fit the relatedness matrix
193 calculated from the full pedigree. To estimate h^2_{GRM1} , the GRM was constructed using an
194 approach that scales by the actual variance in relatedness (approach 3; Robinson et al., 2013).
195 Following de Villemereuil (et al., 2019b), the additive genetic variance (V_A) for each trait was
196 estimated by fitting sex and clutch size as fixed effects and accounting for the random effects
197 of mother, social father, year and month when the nestling was born. Models were run for
198 503,000 iterations with a burn-in period of 3,000, sampling every 10th output from the chain,
199 and convergence checked graphically and using the Heidelberger and Welch convergence
200 test. The heritability was then calculated as the ratio of V_A to V_P (total phenotypic variance),
201 where V_P and thus h^2 accounts for the variance explained by fixed effects (de Villemereuil et
202 al., 2018).

203 Furthermore, to be able to compare our results with the heritability of morphological traits
204 reported in other avian studies (reviewed in Husby et al., 2019), we also used the “preFitModel”
205 function in the R package *RepeatABEL* (Rönnegård et al., 2016). While *RepeatABEL* is mainly

206 known for allowing the inclusion of repeated measures, it was chosen as it allows the inclusion
207 of multiple random effects into the model. The GRM-based heritability (h^2_{GRM2}) was estimated
208 by fitting the same fixed and random effects as above.

209

210 Finally, the package *BayesR* (Moser et al., 2015) was used to infer the phenotypic variation
211 explained by SNPs in different effect size distributions and to estimate the SNP-based
212 heritability (h^2_{BayesR}). The SNP-based heritability differs from the GRM-based heritability as it
213 estimates the proportion of variance accounted for by linear regression on a set of genotyped
214 SNPs (de los Campos et al., 2015). As *BayesR* does not allow for the inclusion of any fixed or
215 random effects, the raw phenotypes were corrected for those effects in *MCMCglmm* (same
216 model as for the heritability analysis, but excluding V_A) and the residuals then used as
217 phenotypes in *BayesR*. We set the mean effect sizes of 0.01, 0.001, 0.0001 or 0 for the four
218 mixture distributions and ran the programme for 50,000 iterations, with a burn-in of 20,000,
219 recording every 10th sample in the chain after that (as suggested in the default settings).

220

221 **Chromosome partitioning of genetic variance**

222 For each chromosome, we estimated the contribution to overall trait variance by fitting a mixed
223 model in *MCMCglmm* with a GRM estimated from markers on the focal chromosome, a GRM
224 calculated from all other markers in the genome, and accounting for sex, clutch size, mother,
225 social father, year and month of birth. As above, the GRMs were calculated using an approach
226 that scales by the actual variance in relatedness. We ran 203,000 iterations with a burn-in
227 period of 3,000, sampling every 10th output from the chain. Due to convergence issues for
228 chromosomes with fewer than 850 markers, we merged multiple chromosomes based on their
229 size according to the zebra finch karyotypes (electronic supplementary material, table S1). The
230 variance was estimated individually for chromosomes 1-14, 1A and 20 and each merged
231 chromosome group.

232 We also used *GCTA* (V1.24, Yang et al., 2011a) to estimate the variance explained by each
233 chromosome. GRMs were generated with the `--make-grm` option for each individual
234 chromosome and all but the focal chromosome. Because the model failed to converge when
235 fitting all autosomes simultaneously, we fit the GRM pairs (e.g. GRM for Chr 1 and GRM for
236 all but Chr 1, using the `--mgrm` option) in the default REML model one by one to estimate the
237 additive genetic variance explained by each chromosome. Both pathways, however, should
238 theoretically result in the same heritability estimate per autosomal chromosome (Yang et al.,
239 2011a). Fixed effects included sex and clutch size, and we adjusted for population stratification
240 using the `-qcovar` function on the top ten principle components.

241

242 For both methods, we used the `cor.test` function in R to test the significance of a positive
243 Pearson's correlation between chromosome size (based on the zebra finch genome) and
244 variance explained. To account for p-value inflation as a result of heteroscedasticity and
245 censoring, we used 100,000 permutations to calculate corrected p-values following the
246 approach of Kempainen and Husby (2018b).

247

248 **GWAS**

249 To test for association between trait and genotype, we used the *RepeatABEL* package. The
250 "preFitModel" function first fits a model without the SNP-effects and computes the variance
251 components for the trait, using the *hglm* package. SNPs are then tested for association by
252 accounting for these random effects in the "rGLS" function in *RepeatABEL*, using the same
253 fixed and random effects as in the heritability analysis. P-values for each SNP are computed
254 using Wald statistics, together with the SNP effect for each marker. We used a Bonferroni
255 correction to control for family-wise error rate from multiple testing (significance value of 0.05
256 divided by the number of markers, giving a critical p-value of 1.26×10^{-6}). All genomic SNP were
257 tested for association, including those that mapped to random or the Unknown zebra finch
258 chromosomes, or were not mapped to a zebra finch chromosome (which we combine as
259 'chromosome 77', electronic supplementary material, table S1). SNPs of interest were further
260 investigated based on the zebra finch genome annotation in the *Ensembl* database (Version
261 100.12: bTaeGut1_v1.p; Cunningham et al., 2019). In addition, we calculated the genomic
262 inflation factor (λ , computed using lower 90 per cent of the distribution), to determine whether
263 population structure was likely to have led to inflation of the test statistics.

264 **3 Results**

265 **i) Trait heritability**

266 The pedigree-based estimates for the heritability of nestling morphological traits tended to be
267 higher than the GRM-based heritabilities, and *MCMCglimm* GRM-based estimates (h^2_{GRM1})
268 were lower than corresponding *RepeatABEL* heritabilities (table 1; electronic supplementary
269 material, table S2, table S3, figure S2). The SNP-based heritabilities from *BayesR* were 0.117,
270 0.079 and 0.076, respectively (electronic supplementary material, table S5). All methods
271 agreed on tarsus length being the 'most heritable' of the three morphological traits.

272

273 **Table 1:** Summary table of the outputs (electronic supplementary material, table S2, table S3) for the trait mean,
274 total phenotypic variance (V_P), pedigree-based and GRM-based heritability (h^2) estimates and additive genetic
275 variance (V_A) for tarsus length (mm), body mass (g) and head-bill length (mm). We report the mean h^2 and V_A for

276 the *MCMCglmm* (ped, GRM1) and *RepeatABEL* (GRM2) estimates for 523 hihi (268 males and 255 females).
 277 Credible / confidence intervals (*MCMCglmm*) or standard error (*RepeatABEL*) are reported in square brackets.

Trait	Mean	V_P	h^2_{ped}	$V_{A, ped}$	h^2_{GRM1}	$V_{A, GRM1}$	h^2_{GRM2}	$V_{A, GRM2}$
Tarsus length	27.576	1.440	0.133 [0.028, 0.253]	0.190 [0.040, 0.362]	0.077 [1.02e ⁻⁹ , 0.154]	0.111 [1.39e ⁻⁹ , 0.219]	0.081 [-0.020, 0.182]	0.082 [0.054, 0.126]
Body mass	37.011	43.662	0.066 [1.02e ⁻¹⁰ , 0.151]	2.880 [4.18e ⁻⁹ , 6.646]	0.042 [2.44e ⁻¹⁰ , 0.105]	1.812 [1.14e ⁻⁸ , 4.548]	0.065 [-0.031, 0.161]	2.050 [1.283, 3.276]
Head-bill length	38.793	2.845	0.120 [5.89e ⁻⁸ , 0.252]	0.343 [1.45e ⁻⁷ , 0.727]	0.032 [5.15e ⁻¹⁶ , 0.094]	0.091 [1.42e ⁻¹⁵ , 0.270]	0.070 [-0.028, 0.168]	0.159 [0.101, 0.251]

278

279 The correlation between off-diagonal elements of the pedigree-based and the GRM-based
 280 relatedness matrices was 0.749 (electronic supplementary material, figure S3). A correlation
 281 of less than one is expected due to factors such as Mendelian sampling and agrees with other
 282 studies (e.g. Pryce et al., 2012; Bérénos et al., 2014; Galla et al., 2020).

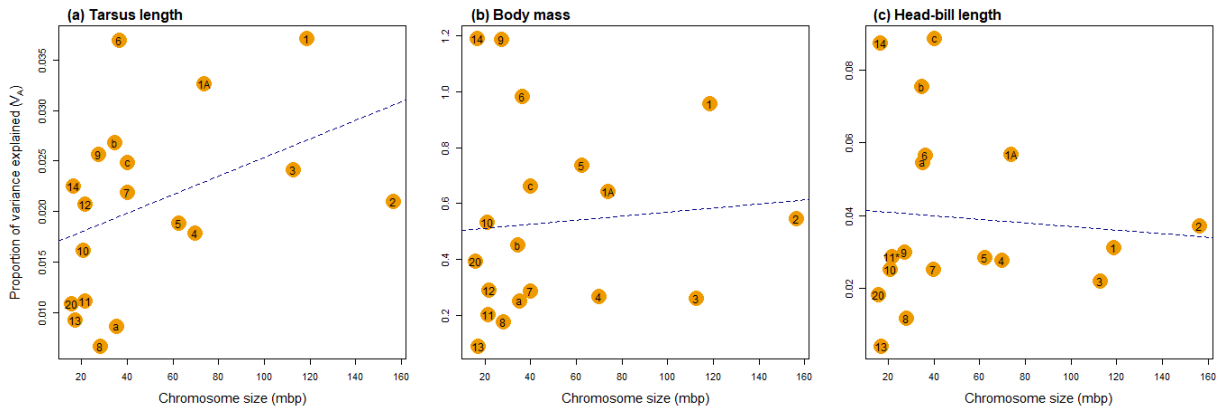
283

284 ii) Chromosome partitioning of genetic variance

285 There was evidence that multiple loci contributed to the variance in all three morphological
 286 traits (figure 1; electronic supplementary material, figure S4). For tarsus length, we found a
 287 positive but non-significant correlation between the proportion of additive genetic variance
 288 explained by each chromosome and the chromosome size (HC corrected p-value = 0.113).
 289 Chromosome 1, 1A and 6 explain the most variation, although the largest chromosome (2)
 290 explained only moderate variation.

291

292 For body mass, we found a weak and non-significant positive correlation between
 293 chromosome size and phenotypic variance explained (HC corrected p-value = 0.422). Here,
 294 chromosomes 14 and 9 explain the most variation.



295
 296 **Figure 1:** The variation explained by each chromosome or chromosome composite (in yellow circles, excluding
 297 'Chr 77') for all three traits (tarsus length, body mass and head-bill length) as calculated in *MCMCglmm*. Plotted is
 298 additive genetic variance explained by each chromosome (V_A) against the length of the chromosome (in Mbp) based
 299 on the zebra finch reference genome. After HC correction, all p-values were non-significant (0.113, 0.422 and
 300 0.574). a = chromosomes 15 and 4A, b = chromosomes 17-19, c = chromosomes 21-28 and 1B (electronic
 301 supplementary material, table S1).

302 Finally, for head-bill length, we found a weak negative correlation between the variation and
 303 the size of the genomic region. The three largest chromosomes explain relatively little of the
 304 overall variation, while chromosome 14, and micro-chromosome composites 'b' and 'c' each
 305 explain the most variation. Chromosome partitioning in GCTA resulted in a comparable picture
 306 for all three traits (electronic supplementary material, figure S5).

307

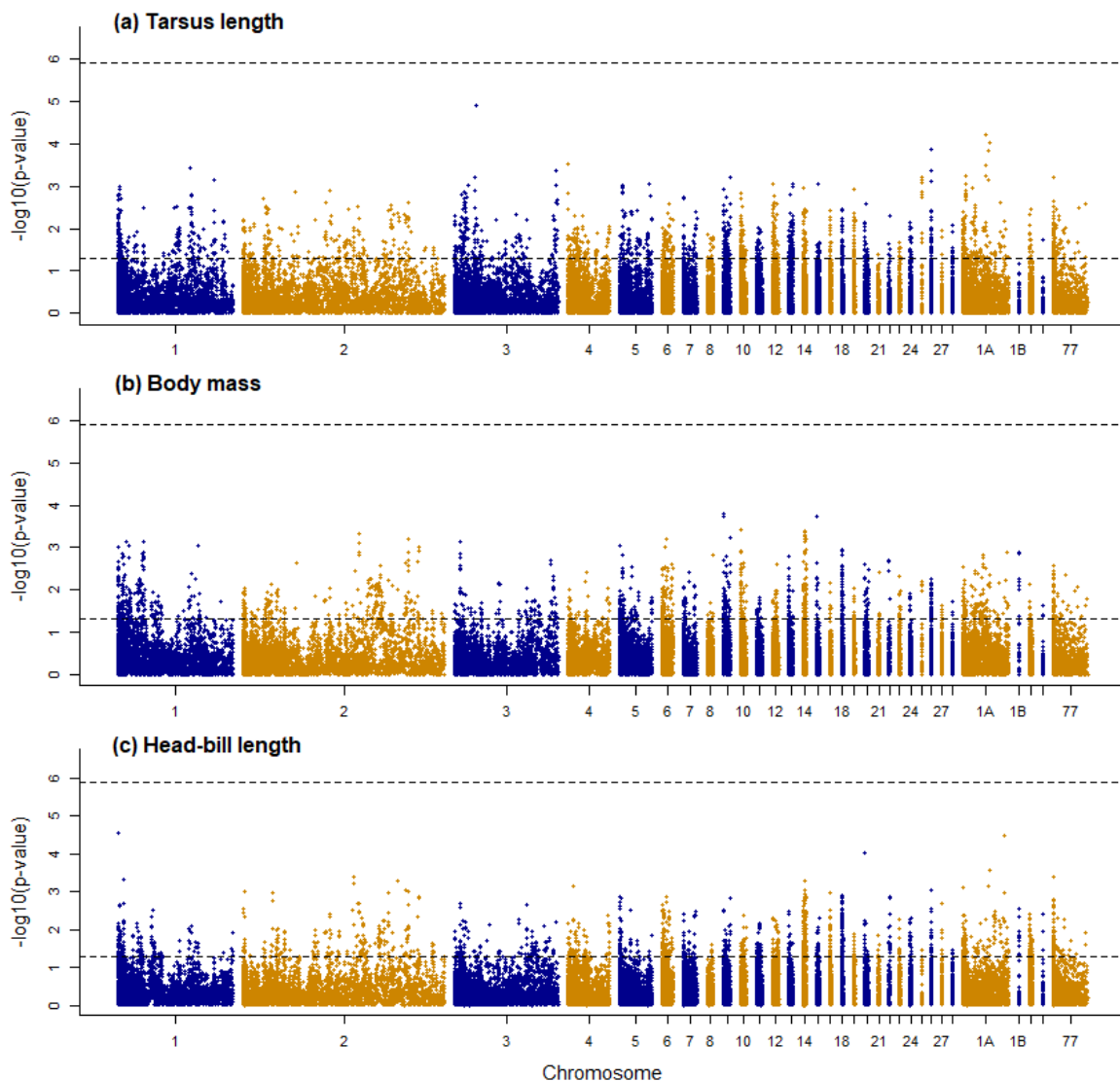
308 **iii) GWAS**

309 No SNP was significantly associated with the morphological traits after testing for association
 310 in *RepeatABEL* (figure 2). According to the well-curated zebra finch genome (which the hihi
 311 chromosome assignment is derived from), the SNP with the highest support for association
 312 with tarsus length is located in one of the intronic regions of the *HEY2* transcription factor
 313 (electronic supplementary material, figure S6), which is involved in cardiac morphogenesis,
 314 neurogenesis and somitogenesis (Ihara et al., 2020).

315 For head-bill length, there are two SNPs that, while not significant, show a stronger association
 316 with the trait than all others. One of them is placed in an intron of an uncharacterized protein-
 317 coding gene (ENSTGUG00000006844) on chromosome 1, the other in between two genes
 318 associated with cat eye syndrome (CECR1, CECR2) on chromosome 1A. The third most
 319 associated SNP for head-bill length was found on chromosome 20, a chromosome previously
 320 associated with bill length (Knief et al., 2012; Silva et al., 2017).

321 The top ten SNPs with the lowest p-values across all traits can be found in electronic
322 supplementary material, tables S4. We refrained from calculating the additive genetic variance
323 explained by each of the most associated SNPs, as p-values were generally high. The genomic
324 inflation factor values ranged from 0.96 to 1.05 for all three phenotypic traits under investigation
325 (electronic supplementary material, figure S7), suggesting that population structure has been
326 adequately accounted for.

327



328

329 **Figure 2:** Manhattan plots for the association between single nucleotide polymorphisms and (a) tarsus length, (b)
330 body mass and (c) head-bill length in 523 hihi nestlings of Tiritiri Matangi. Top black dashed line: Bonferroni
331 significance threshold, bottom dashed line: nominal significance ($p\text{-value} = 0.05$). A description of the chromosome
332 order and naming can be found in the electronic supplementary material, table S1.

333 The *BayesR* analyses confirmed that all traits under investigation appear polygenic, with less
334 than 9% of the SNPs affecting trait variation, the majority of which contributed to the smallest
335 effect size distribution (electronic supplementary material, table S5). A comparison of the
336 SNPs with the lowest p-values from the GWAS showed reasonable agreement with those
337 inferred to have a high probability of inclusion in the largest effect size distribution from *BayesR*
338 (electronic supplementary material, tables S4).

339

340 **4 Discussion**

341 Adaptive response requires that there is genetic variation in traits linked to fitness, and further,
342 that response to selection is unconstrained by trade-offs with other traits and loci. Here, we
343 examined the genetic basis of variation in nestling tarsus length, body mass and head-bill
344 length in the hihi, a threatened yet well-monitored bird species of Aotearoa | New Zealand, in
345 order to understand whether these traits can respond to current and future selection pressures.
346 Our analyses confirmed low heritability estimates, and support a polygenic basis for all three
347 traits, with no loci of large effect contributing to trait variation. Given the low effective population
348 size and the polygenic architecture, such that adaptive variation lost via drift is unlikely to be
349 replenished by mutation in the short-term, we conclude that evolutionary potential in hihi is
350 likely constrained even further than the already low heritabilities suggest.

351 We have three reasons to believe that a polygenic architecture underlies all three
352 morphological traits under investigation: (i) for tarsus length, the variance explained by each
353 chromosome scaled with chromosome size, and for all traits more than one chromosome
354 contributed to variation, (ii) there were no significant SNP associations in the GWAS and (iii)
355 the majority of loci explaining variation are of small effect size. This study is in good agreement
356 with studies in other passerines, (e.g. great tits (Santure et al., 2013; 2015; Gienapp et al.,
357 2017a; Bosse et al. 2017; Kim et al., 2018), collared flycatchers (Husby et al., 2015; Kardos et
358 al., 2016; Silva et al., 2017), house sparrows (Silva et al., 2017; Lundregan et al., 2018), great
359 reed warblers (Hansson et al. 2018), reviewed in Husby (et al., 2019)) as well as studies from
360 other wild animal populations (e.g. Bérénos et al., 2015), where many loci with small effect are
361 responsible for variation in adaptive phenotypes, rather than a few large-effect loci.

362 Our pedigree- and GRM-based heritability estimates support a small but non-zero heritability
363 for all traits and agree with previous pedigree heritabilities estimated from a much larger
364 number (>2,000) of individuals (de Villemereuil et al., 2019b). Interestingly, the GRM-based
365 heritabilities were lower than the pedigree-based estimates, although the credible intervals are
366 overlapping in all cases. One reason for this may be that our SNP array is not dense enough

367 to have captured true genome sharing more accurately than the pedigree has done. Given the
368 number of SNPs genotyped, this seems unlikely (Berenos et al., 2014), although we note that
369 we have not successfully genotyped SNPs from a number of microchromosomes, including
370 chromosome 16. We acknowledge that, while 523 individuals is a large dataset for a
371 threatened species, our sample size is likely to be somewhat limiting and has certainly
372 contributed to the large credible intervals and standard errors around both the pedigree- and
373 GRM-based heritability estimates, which may also explain some of the differences between
374 estimates. Interestingly, a study of Corsican blue tits (*Cyanistes caeruleus*; Perrier et al., 2018)
375 suggests that a sample size of around 500 birds and data from a 50K SNP chip can outperform
376 heritability estimates from a seven-generation pedigree. The ability to estimate heritabilities
377 without long-term and near-complete monitoring of a population offers exciting opportunity to
378 infer adaptive potential in other wild populations and will be particularly valuable in assessing
379 the vulnerability of endangered species (Gienapp et al., 2017b). The sample size, number of
380 markers and low heritabilities is also likely to have limited power to detect association in the
381 GWAS analyses. Linkage disequilibrium between neighbouring markers is moderate (mean of
382 0.518, calculated with all 523 individuals using plink --r2 and reporting all pairwise values with
383 --ld-window-r2 0.0) and suggests that power to detect a locus explaining *all* of the trait
384 heritability ranges from 0.306 to 0.516 (Wang and Xu 2019; electronic supplementary
385 material, table S6). As expected, nestling mass, with the lowest heritability due to substantial
386 maternal and month effects compared to the other traits (de Villemereuil et al. 2019b), has the
387 lowest power to detect association.

388 Morphological traits are often the subject of wild bird studies, as they can reveal evolutionary
389 patterns over time and in turn shed light on the adaptive response of traits known to correlate
390 with fitness (Merilä and Sheldon, 2001; Gardner et al., 2011). The three morphological traits
391 studied here are all under selection as previous analyses suggest that the population mean of
392 all three traits is substantially smaller than the fitness optimum (de Villemereuil et al. 2019b)
393 and that there is a trade-off between body size and agility mediated during the highly energetic
394 breeding season of the hihi (Low 2006). Although there was no significant association detected
395 in the GWAS, it is interesting to note the regions that were most highly associated with tarsus
396 length and head-bill length. The tentative association of a locus on chromosome 3 with tarsus
397 length and of variants on chromosomes 1 and 1A with head-bill length does not appear to be
398 replicated in some studies of passerine body size (Silva et al., 2017; Bosse et al., 2017), while
399 Lawson and Petren (2017) highlight a region on chromosome 1A in increased linkage with
400 regard to beak shape in Darwin finches (*Geospiza*). Additionally, the third-highest association
401 for head-bill, a SNP on chromosome 20, stands within an intron of NFS1 cysteine desulfurase

402 and is 7-12Mb distant from QTLs previously associated with beak morphology (Knief et al.,
403 2012; Silva et al., 2017). The challenges in identifying significant loci in a genome-wide
404 association analysis include low SNP density and small sample sizes (Santure & Garant 2018),
405 both of which are likely to apply to the hihi study system to some degree. All the above-
406 mentioned loci are interesting candidates for further study if sample size and SNP density in
407 these regions can be increased. Creating a larger sample size through combining all monitored
408 populations could improve the precision of estimates, and shed more light on the effects of
409 translocations on trait variation and responses to selection.

410 Despite the low heritabilities of the traits, our genomic analyses have provided important further
411 insights for hihi, and threatened species more generally. A more nuanced understanding of the
412 genomic architecture of a trait under selection helps assess the likely speed of the adaptive
413 response in a species and the longer-term total response to selection (John & Stephan, 2020;
414 Höllinger et al., 2019). We have demonstrated that many loci of small effect are likely to
415 contribute the majority of variation to all three morphological traits. Given the small effective
416 population size of all hihi populations, many adaptive variants are likely to be lost to drift at a
417 rate that is faster than the input of new variation via mutation (reviewed in Johnson & Barton,
418 2005; Willi & Buskirk, 2006). This is likely to slow the speed of adaptation and lead to further
419 constraint on the adaptive potential of the species. Most importantly, other wild populations
420 may be facing similar threats as the hihi, highlighting the need for continued collection and use
421 of genomic resources to aid conservation measures. We therefore encourage further
422 characterisation of the heritability and genomic architecture for species that face anthropogenic
423 threats including habitat decimation and climate change, in order to better predict their
424 evolutionary potential to adapt to such challenges (Gienapp & Brommer, 2014). Finally, we
425 acknowledge that future studies will need to go beyond describing quantitative parameters in
426 order to successfully combine genomic insights with active species conservation, including
427 informing management actions that can buffer species with small population sizes from the
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429

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451

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