Little adaptive potential of a threatened passerine

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Summary

Threatened species face numerous threats, including future challenges triggered by global change. A possible way to cope with these challenges is through adaptive evolution, which requires adaptive potential. Adaptive potential is defined as the genetic variance needed to respond to selection and can be assessed either on adaptive traits or fitness [6]. However, a lack of high quality data has made it difficult to rigorously test adaptive potential in threatened species, leading to controversy over its magnitude [6–8]. Here we assess the adaptive potential of a threatened New Zealand passerine (the hihi, *Notiomystis cincta*) based on two populations: *(i)* the sole remaining natural population, on the island of Te Hauturu-o-Toi and *(ii)* a reintroduced population with a long-term dataset (intensively monitored for 20 years) based on the island of Tiritiri Matangi. We use molecular information (reduced representation genome sequencing, on both populations), as well as long-term phenotypic and fitness data from the Tiritiri Matangi population to find: *(i)* a lack of molecular genetic diversity at a genome-wide level in both populations, *(ii)* low heritability of traits under selection and *(iii)* negligible additive genetic variance of fitness in the Tiritiri Matangi population. In combination, these results support a lack of adaptive potential in this threatened species. We discuss our findings within the context of other passerines and methods for assessing adaptive potential; and the impact of these results on conservation practice, for the hihi and species of conservation concern in general.

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Results

The hihi (*Notiomystis cincta*, Du Bus, 1839) is an endemic New Zealand bird and the sole representative of the *Notiomystidae* family [9]. Once common across the North Island, it is now naturally occurring only on the island of Te Hauturu-o-Toi (Little Barrier Island, population estimated around $1,000$ - $3,000$ individuals). As part of a concerted conservation effort, the hihi has been successfully reintroduced to six mammalian predator-free sanctuaries, one of which is situated on the island of Tiritiri Matangi (founded in 1995 by 53 reintroduced individuals and currently stable at around 100-175 breeding individuals). Since its establishment all breeding events have been intensively monitored, and every individual is systematically sampled and nestlings banded and morphological traits measured [10], making this one of the largest and most comprehensive long-term datasets of any threatened species. There is no dispersal in or out of either of the studied islands.

Molecular diversity

Genomic sequence data from restriction site associated DNA (RAD) sequencing, a form of reduced representation genomic sequencing, revealed low genetic diversity in hihi. Sequence data was derived from 26 individuals from Te Hauturu-o-Toi and five individuals from Tiritiri Matangi populations. The proportion of polymorphic sites within the RAD sequences was 0.36% (SE ± 0.0091%) using both populations. Polymorphic sites were scarcer in Tiritiri Matangi (0.22%, SE ± 0.0048%), when compared to the source population of Te Hauturu-o-Toi (0.34%, SE ± 0.0047%), which might originate from the smaller sample size in Tiritiri Matangi, and/or the bottleneck [11] when Tiritiri Matangi was established. The nucleotide diversity (π) was estimated at 0.00095 (SE ± 4.64×10^{-6}) and was also lower in Tiritiri Matangi (0.00088, SE ± 4.35×10^{-6}) than in Te Hauturu-o-Toi (0.00095, SE ± 1.40×10^{-5}). Both of these molecular diversity metrics were lower than any comparable (i.e. 'sequence-based') estimates for passerine birds we have been able to identify in the literature [see Figure 1; 1–5]. Note that while the only threatened species (the Florida scrub-jay) included in our comparison has the lowest nucleotide diversity of the other species, the hihi still has a diversity 2.6 times lower.

Quantitative genetics of traits

We performed a quantitative genetics analysis on eleven commonly measured avian traits that were either directly measured or derived from the pedigree data in Tiritiri Matangi: three morphological traits (mass, tarsus length and head-to-bill length, all measured at 21 days of age, just before fledging), three life-history traits (longevity, probability of being recruited and age at first reproduction) and

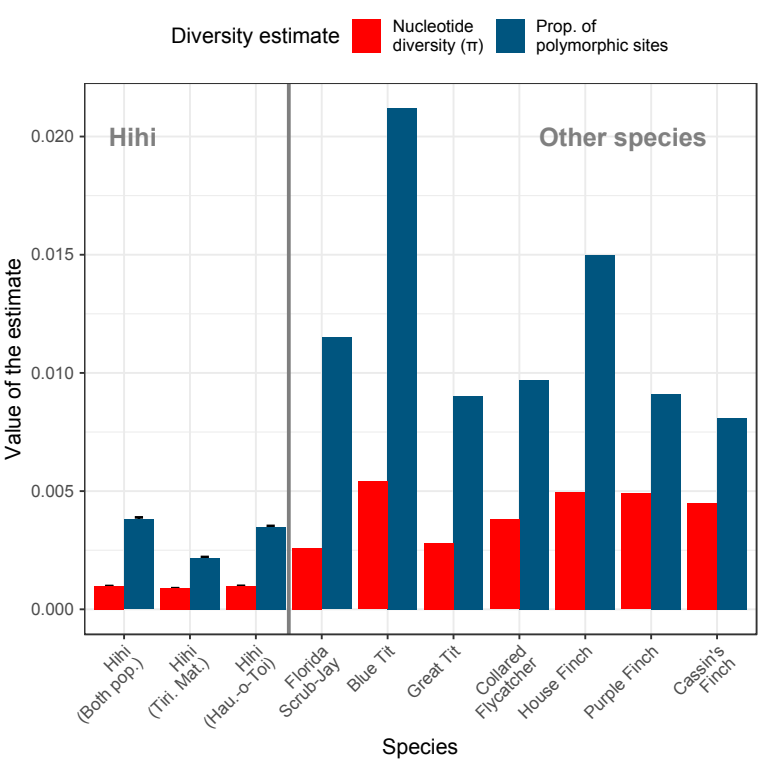

Figure 1: Nucleotide diversity (π, red) and proportion of polymorphic sites (blue) for the hihi populations (Both pop: Both populations; Tiri. Mat.: Tiritiri Matangi; Hau.-o-Toi: Te Hauturu-o-Toi) along with estimates for other species (Florida scrub-jay *Aphelocoma coerulescens*, blue tit *Cyanistes caeruleus*, great tit *Parus major*, collared flycatcher *Ficedula albicollis*, house finch *Haemorhous mexicanus*, purple finch *Haemorhous purpureus*, Cassin's finch *Haemorhous cassinii*) identified in the literature [N. Chen, pers. comm. for the Florida Scrub-Jay data, C. Perrier, pers. comm. for the tit data, 1–5]. Nucleotide diversity for the collared flycatcher is a weighted average of autosomes and the Z-chromosome estimates from [5], considering the latter represents 6% of the genome. Error bars symbolising the standard error (computed using leave-one-out jackknife on the individuals) are provided for the hihi only. See more information on individual heterozygosity in Figure S1.

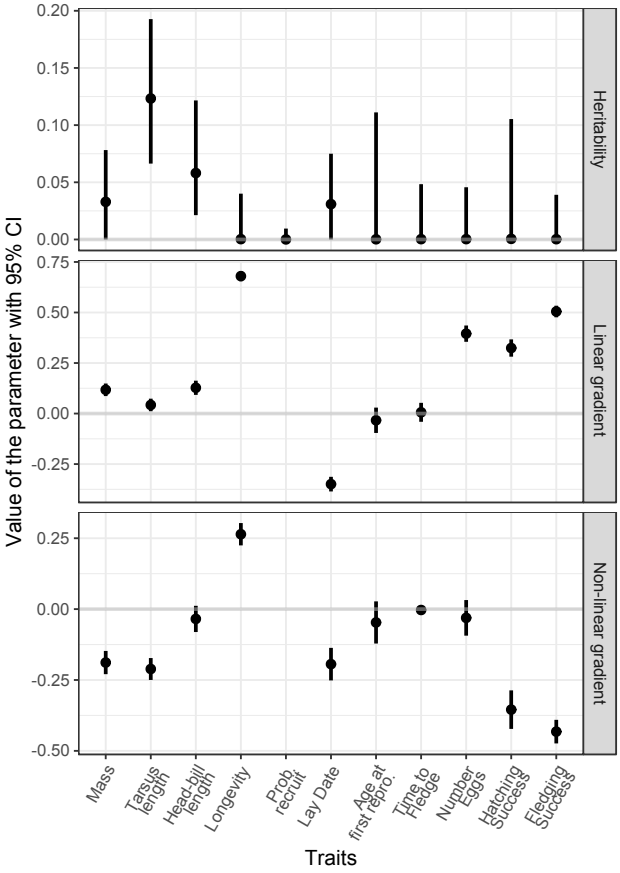

Figure 2: Point estimate and 95% credible/confidence intervals (CI) of the heritability (top-panel, point estimate is the posterior mode), linear (middle-panel) and non-linear standardised selection gradients (bottom-panel) for each of the studied traits. The selection gradients for the probability of recruitment are not calculated, as the fact that all non-recruits have a fitness of 0 creates numerical complications. Sample sizes are (from left to right) 2,098, 2,098, 2,098, 2,288, 2,183, 1,371, 581, 1,259, 1,383, 1,358 and 1,244. More details are available in Figure S2, Figure S3, Table S1, Table S2 and Table S3.

five breeding traits (lay date, number of eggs laid, hatching and fledgling success and time from egg laying to fledgling). To identify the traits under selection, we computed the linear and non-linear selective gradients [12, 13]. We then used 'animal models', a type of generalised linear mixed model, and pedigree information, accounting for confounding effects and using appropriate statistical distributions for each trait, to compute the additive genetic variance (V_A) and heritability (h^2) of all the traits. Estimates are provided on the observed data scale, *sensu* [14]. Finally, we tested the ability of the sample size of our dataset to correctly estimate a *small, but substantial* signal for the adaptive traits and fitness with a simulation analysis. Detailed results are available in Table S1, Table S2 and Figure S3 of the Supplementary Information.

Selection gradients All traits but time to fledge and age at first reproduction were under selection, with a significant linear and/or non-linear standardised selection gradient (Figure 2 and Table S1). The linear selection gradient was positive for all traits except for lay date (and age at first reproduction, although the linear gradient was not significant) for which a negative selection gradient was estimated. Except for adult longevity, all non-linear selection gradients were negative, which suggests stabilising selection is operating, particularly when the parameter was significant.

Quantitative genetics of the studied traits Estimated posterior modes for heritabilities and additive genetic coefficients of variation of the three morphological traits were small (Figure 2, see also Table S2 and Figure S2, fledgling mass: $h^2 = 0.0329$, additive genetic coefficient of variation (CV_A) = 3.5%; tarsus length: $h^2 = 0.123$, $CV_A = 1.7\%$ and head-bill length: $h^2 = 0.0581$, $CV_A = 1.2\%$). In contrast to the morphological traits, all but one (lay date) life-history and breeding traits had an estimated posterior mode for the heritability below 10^{-3} (see Figure 2, Table S2 and Fig-

Table 1: Quantitative genetic analysis of fitness (lifetime reproductive success) using a zero-inflated Poisson. The results are shown separately for the zero-inflated (binomial) and the Poisson part of the model. All statistics (Pop. Mean : population mean, V_P: phenotypic variance, V_A: additive genetic variance, h^2: heritability and I_A: evolvability) were computed on the observed data scale. Estimates are reported as posterior mode (posterior median) [95% credible interval].

Model part	Pop. Mean	V_P	V_A	h^2	I_A
Zero-infl. part	0.759 (0.76)	0.183 (0.18)	1.29E-5 (6E-4)	0.00011 (0.0033)	3.21E-5 (0.001)
	[0.68 – 0.82]	[0.15 – 0.22]	[1.4E-11 – 0.0038]	[7.8E-11 – 0.022]	[2.3E-11 – 0.0065]
Poisson part	8.14 (8.4)	151 (146)	0.0078 (0.73)	4.97E-5 (0.0045)	0.000587 (0.01)
	[5.9 – 12]	[49 – 479]	[2.3E-10 – 5.7]	[1.3E-12 – 0.034]	[3E-12 – 0.077]

ure S2). These extremely low estimates of heritability were driven by very low estimated values of the additive genetic variance, with all lower bounds of the 95% credible interval below 10^{-7}. Although the signal for a non-zero additive genetic variance for lay date was stronger, the point estimate (posterior mode $h^2 = 0.0289$) was still extremely low and the 95% credible interval lower bound close to zero (see Table S2). Our simulation analysis (Figure S3) demonstrated our data structure would be able to detect heritabilities as low as 0.1 with confidence for these traits.

Additive genetic variance of fitness

The most direct measure of adaptive potential is the additive genetic variance of fitness. Here, we used an animal model with a zero-inflated Poisson distribution to compute the additive genetic variance of lifetime reproductive success of the individuals. This analysis suggests a very low adaptive potential in hihi, with extremely small additive genetic variances of both the zero-inflated and Poisson component of our model, resulting in extremely small heritabilities and evolvabilities (see Table 1). The zero-inflated component had a relatively large population mean of 0.759, which agrees well with the observed probability of not being recruited into the breeding population (0.724). Our simulation analysis (see Figure S3) placed these estimates rather below what would be considered a *small, but substantial* signal for additive genetic variance of fitness. They also demonstrated an upward bias in the posterior median estimation for the evolvability of the Poisson part, meaning that the true value of I_A is likely to lie between the posterior mode (0.000587) and posterior median (0.01) shown in Table 1.

Discussion

Lack of adaptive potential in the hihi in comparison to other species The three measures of adaptive potential we employed here all support a lack of general and adaptive genetic diversity in the hihi. The levels of molecular genetic diversity found in this study are consistent with a depleted diversity compared to other passerine species (Figure 1). Comparing individual heterozygosity in pairs of threatened and non-threatened bird species, [15] similarly found a diversity roughly two to five times lower in threatened birds. Our average level of individual heterozygosity (0.00069, see Figure S1) falls well within the range observed by [15] for threatened bird species (0.0004 – 0.00091). More generally, when compared to estimates available in the database from [16], our nucleotide diversity seems typical of threatened species (average nucleotide diversity of 0.00115 for the 33 species classified as vulnerable, endangered or critically endangered), although the majority of such species are not passerines and might differ due to other features (such as overall fecundity) given the larger phylogenetic divergence. All of the traits studied here displayed small to inconsequential levels of additive genetic variance and heritability. The analysis of two morphological traits (tarsus length and head-bill length) yielded small heritabilities, with two other traits (body mass and lay date) having at best ambiguous support for heritabilities away from zero. The life-history and breeding traits (except lay date) were all characterised by extremely low posterior heritabilities, with all below a value of 0.05. Our simulation analysis confirms that our sample size is sufficient to accurately estimate low heritabilities, and that the majority of our heritabilities are well below what might be considered *small, but substantial* ($h^2 = 0.1$), and hence that adaptive potential is very limited. Furthermore, these estimates remain extremely low in the context of typical heritability values for other passerines found in the current literature. For example, using bird species data available from [17] to compute an average heritability value, we found estimates larger than those found in this study for body mass (h^2 = 0.42, 98% of the values above ours), tarsus length (h^2 = 0.59, 96%) and lay date (h^2 = 0.149, 90%). The additive genetic variance and related standardised measures were also very small for fitness. Only two other studies [18] used non-Gaussian animal models to measure and report the additive genetic variance of fitness [19, 20]. While they report variances on the latent scale, this is equivalent to our evolvability estimate [14]: the estimates for song sparrow (*Melospiza melodia*) of [20] are considerably greater than ours (1.72 for males and 2.01 for females), while the estimates for American red squirrel (*Tamiasciurus hudsonicus*) of [19] are more comparable to ours (from 0.004 to 0.017, but note that their credible intervals have much higher lower and upper bounds). While the additive genetic variance of relative fitness is more related to an *instantaneous* rate of adaptation, rather than a potential for *future* adaptation, it is one of the best proxies to predict the ability of a species to face environmental challenges [18, 21], especially for threatened species such as hihi that are already shifted away from their optimal habitat [22]. The lack of adaptive potential and reduced genetic diversity are likely to be as a result of the bottleneck experienced during the collapse of the species, as well as

an extended period where the species consisted of a single population. The hihi was widespread across the North Island of New Zealand before its rapid decline following European colonisation with extirpation from the "mainland" by 1885 [11]. The range of the species was then restricted to a sole surviving population on Te Hauturu-o-Toi (3083 Ha) for more than a century. Despite changes in population size on Te Hauturu-o-Toi, hihi are thought to have retained a relatively stable level of genetic diversity. This is primarily due to the largely promiscuous mode of mating of the hihi, with an extremely high rate of extra-pair paternity [23, 24] and high natal dispersal [25]. The remnant population is also currently estimated to be large and stable [26] and no trace of a recent bottleneck was found using microsatellite data [11]. However, information about past hihi diversity and the composition of Te Hauturu-o-Toi at the time of collapse are unfortunately harder to infer.

Relationship between molecular genetic diversity and adaptive potential The lack of molecular genetic diversity in the hihi differs from previous conclusions of [11], who found a high genetic diversity, based on microsatellites, in the reintroduced populations and a small reduction in genetic diversity following reintroduction events. This difference is likely because [11] report *relative* rather than *absolute* diversity, i.e. here we include monomorphic sites. Our approach allows for a direct between-species comparison of the levels of polymorphism and related *per-site* nucleotide diversity. Nucleotide diversity will also be relatively slow to recover from erosion due to a low per-nucleotide mutation rate compared to microsatellite markers. In contrast, microsatellites provide information on the distribution of polymorphism (i.e. population structure) rather than its actual level (although number of alleles can be used for this, genotyped microsatellites are usually chosen to be polymorphic in the first place, leading to ascertainment bias, [27]). Average *per-site* nucleotide diversity is also a more convincing molecular proxy of adaptive potential, being closer to the definition of the *per-locus* additive genetic variance [28]. A meta-analysis [29] demonstrated little connection between adaptive potential and diversity at molecular markers, which we suggest may be an artifact of ascertainment bias (e.g. using *relative*, rather than *absolute*, diversity). While relative diversity might still be a useful measure to study e.g. inbreeding issues [30–32], we thus advocate for the use of diversity estimates based on sequence polymorphism as these are more closely related to adaptive potential.

Consequences for conservation Our results suggest that hihi currently do not have the adaptive potential to evolve in response to the environmental threats the species is exposed to (and, possibly, will be exposed to in the future), despite strong signs of selection detected for all but two studied traits (see Figure 2 and [22] for a detailed analysis of lay date). Despite this, most hihi populations are currently showing good demographic health with supportive management (including predator control and supplementary feeding). In particular, the hihi population on Tiritiri Matangi is one of the most productive and currently being used as a source of birds for translocation to other populations [10]. However, our results raise a number of questions about the future management of threatened populations. A fundamental objective of conservation management is to maximize the number and size of populations as quickly as possible, thus avoiding heightened extinction risk stemming from stochastic processes in small populations. Recovering species whose populations have become chronically small will likely face similar problems of low adaptive potential, as we have reported for the hihi. However, the management objective of increasing population size and numbers will ultimately also create conditions which will allow regeneration of adaptive potential, albeit over a long period of time. Theoretical models indicate that the level of genetic variance recovered over time will depend on the population effective size N_e, although the time to equilibrium is also of N_e generations [33, 34]. A reassuring property of the regeneration of genetic variation, however, is that it is gradual (what is gained is gained, as long as conditions are stable) and quicker at the beginning of the process [33]. Nonetheless, when managing rapid recovery from small population sizes, conservation managers should be cautious of the unintentional genetic legacy effects that may ultimately reduce population viability; i.e. increased inbreeding and genetic drift. Both inbreeding accumulation and drift may be promoted, for example, by prioritising a few prolific breeders in a chronically small population in contrast to managing for wider founder representation [35, 36]. The costs and benefits of alternative recovery strategies (combining genetic and non-genetic elements) should be projected as probabilistic extinction risks at suitably long time scales to optimize management effort. Once populations have been recovered to a larger size they will still likely require management support to protect or mitigate environmental pressure in until such time as adaptive potential has been restored.

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Author contributions AWS and PB conceived of the study. JGE developed the demographic dataset and supervised the data collection. PB developed the microsatellite dataset, supervised the genotyping and performed the pedigree reconstruction. KDL performed the genome assembly. PdV designed and conducted the analysis of the data, with advice from all other authors. PdV wrote the paper, with input from all other authors.

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Declaration of Interests The authors declare no competing interests.

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STAR⋆Methods

Contact for reagent and resource sharing

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Pierre de Villemereuil (bonamy@horus.ens.fr).

Experimental model and subject details

The remnant hihi population on Te Hauturu-o-Toi (Little Barrier Island; 36°12'S, 175°05'E) is largely left unmanaged beyond ensuring the island stays mammalian predator free. Monitoring is limited. In contrast, the hihi population on Tiritiri Matangi (36°36'S, 174°53'E) is intensively managed and has been closely monitored since its establishment in 1995. All birds are individually identifiable (colour rings added almost exclusively as nestlings) and all nesting attempts known, as hihi nest almost exclusively in nest boxes. Dispersal is not possible between the populations. Hihi feed on a mix of fruits, nectar and small invertebrates [37], but are also provided with supplementary food (20% by mass sugar water) on Tiritiri Matangi. Hihi usually reproduce in their first year, during the austral spring and summer [September to February, 37]. Females lay clutches ranging from three to five eggs and can produce multiple clutches within a season although normally only one or two are successful. Despite males providing around 30% of the care during rearing [38], extra-pair paternity in this species is widespread. Around 60% of chicks within a brood are sired by extra-pair males [39]. Prior to whole genome and RAD sequencing, hihi blood samples stored in ethanol were extracted at the Natural Environmental Research Council Biomolecular Analysis Facility (Sheffield, United Kingdom) using an ammonium acetate protocol [40], and quantified using a DNA fluorometer (Hoe-

fer DynaQuant200) after being assessed for quality on an agarose gel.

Method details

Pedigree reconstruction Systematic blood-sampling and genotyping at 18 microsatellite markers and two sex-specific loci for Tiritiri Matangi individuals began in 2004 [41], allowing us to reconstruct the paternities accounting for possible extra-pair copulations, following [42]. Because blood sampling was only initiated in the 2003/2004 breeding season, information relating to the genetic sire of individuals born previously is missing. For these individuals, we considered the information as missing, rather than using the social sire.

Genome assembly Low coverage whole genome sequencing of ten birds (a subset of the samples used in RAD sequencing below) was used to assemble a draft hihi genome, with seven of the samples sourced from Te Hauturu-o-Toi and the remainder from Tiritiri Matangi. Samples were multiplexed and two PCR-free DNA libraries were prepared by New Zealand Genomics Limited and used to generate 100bp paired-end illumina reads over two lanes of Illumina HiSeq™ sequencing. This resulted in a total of 879,894,554 reads with a median of 89,508,541 per sample. Sequence quality was assessed using FastQC [**andrews_fastqc_2014**]. Adapters and poor quality reads were removed with Trimmomatic-0.33 [43] under strict conditions (ILLUMINACLIP:TruSeq3-PE-2.fa:2:30:10, LEADING:3, TRAILING:3, SLIDINGWINDOW:4:20, MINLEN:70, CROP:110); over-represented reads identified in FastQC were also removed by appending them to the TruSeq3-PE-2.fa file. Sample 10, an individual from Te Hauturu-o-Toi and the sample with the most reads (126,397,278), was used to run SOAPdenovo2 version 1.5.14 [44] at kmer sizes ranging from 25 - 95. The optimum kmer length of 36 bases was determined by N50 value and length of assembly and was used in subsequent assemblies. Each of the samples was assembled using SOAPdenovo2 with kmer 36 and insert size 210. Following assembly, sample 10 was also assessed to have the highest quality assembly, with a total genome size of 1,002,019,675 bases, an N50 of 1928, and 3,024,992 contigs ranging in length from 32 to 24,819 bases, and consequently this assembly was chosen as the draft hihi reference genome.

RAD sequencing We used RAD sequencing [45] to obtain sequences from 26 individuals from Te Hauturu-o-Toi and 5 individuals from Tiritiri Matangi. The unbalanced sampling is explained by the fact that the RAD sequencing was initially used to detect SNPs to design a SNP chip, targetting the diversity in the Te Hauturu-o-Toi source population. Samples were standardised to $\simeq$ 50ng/uL and then sent to Floragenex Inc. for RAD sequencing following Baird et al. [45]. Extracted DNA was digested with SbfI, and barcodes and adaptors ligated. One sample was replicated, and a *Saccharomyces bayanus* (yeast) control sample included, for a total of 33 independently indexed libraries. Libraries were pooled for sequencing across two lanes of Illumina HiSeq 2000, generating 257,833,998 reads of 101 bases. The reads were processed and cleaned using the STACKS software [46] (`process_radtags` with options `-e sbfI -c -q -s 15`). They were then mapped on the reference genome (Sample 10 above) using the `mem` procedure of the Burrows-Wheeler alignment tool [`bwa` with default options, 47]. Alignements were filtered for being mapped with a quality of 60. They were then stacked using the `mpileup` of the `bcftools` suite [again using default parameters 48]. Sites with phred-scale quality scores below 20 were discarded. Individual genotypes with read depth below 10 or allelic depth below 5 were considered as missing. Only sites without missing values were kept for each of the subsamples (total: 753,920 sites; only Te Hauturu-o-Toi: 855,845 sites; or only Tiritiri Matangi: 2,793,318). Our results however are unchanged when using only sites with no missing values or allowing 30% of missing values (maximum relative change of 5%).

Phenotypic information During the breeding season, all active nest boxes are inspected every few days. The identity of the occupying male and female are recorded, along with the date of egg laying, hatching and fledging, with the corresponding number of eggs/chicks each time. We used these data to derive the number of eggs laid, lay date, hatching success (proportion of eggs hatched), fledging success (proportion of fledged individuals) and time to fledge (time elapsed between lay date and fledging date). Nestling morphological measures are taken (mass, tarsus length and head-to-bill length) and individuals colour-ringed at 21 days of age. We used the pedigree information to derive the following life-history traits: probability of being recruited into the breeding population, adult longevity (number of years survived after the first breeding season post-fledging), and age at first reproduction. Fitness was measured as the total number of offspring fledged over an individual's lifetime (lifetime reproductive success, 0 for individuals that never bred) or from each clutch (number of fledglings) for the breeding data. We removed individuals that were still alive and breeding, and therefore without a complete lifetime reproductive success measure, from the individual fitness measures. Individual and breeding data were collected from the 1995/1996 to 2014/2015, and from the 1997/1998 to 2014/2015 breeding seasons respectively.

Quantification and statistical analysis

Genetic analysis From the RAD sequencing data, the average *per-site* nucleotide diversity and proportion of polymorphic sites were computed using VCFtools [49].

Quantitative genetics models To estimate quantitative genetic parameters on the Tiritiri Matangi population, we used the phenotypic and pedigree information to run generalised linear mixed models, known as animal models, using the R package `MCMCglmm` [50]. For all traits, the fixed effects of sex, fledgling mass (for individual data, i.e. morphological and life-history traits), clutch number in the season, dam age and laying date (for breeding data) and clutch size (for both) were, when relevant, tested for significance (using the `pMCMC` value inferred by `MCMCglmm`) and included in the mixed model if significant (see Table S2). For the individual traits, random effects included additive genetic effect (using the pedigree information), identity of the dam, identity of the social sire, year (i.e. year corresponding to the start of breeding season) and month of birth. For the breeding traits, random effects included additive genetic effect, identity of the breeding female (accounting for repeated measures), identity of the male mate and year (see above) of the breeding event. The error distributions (Gaussian, Poisson with a log link, or binomial with a probit link) were chosen to fit each trait (see Table S2). The number of iterations and the thinning interval were chosen for each model so as to ensure that the MCMC effective sample sizes for all parameters of the model were above 1,000; and were increased if this was not the case. As a result, our minimum MCMC effective sample size is 2,130. Burn-in was set to a minimum of 3,000 iterations and convergence was checked using the Heidelberger and Welch [51] convergence test as implemented

in the coda R package [52]. Burn-in was increased until convergence was reached. Whenever possible, we performed father-son, mother-daughter and parent-offspring regressions. For breeding traits, only mother-daughter regressions were possible. Such regressions were not possible for the probability of recruitment and hatching and fledgling success, as there was no variation in the parent population since, by definition, all were recruited. Finally, we performed a quantitative genetic analysis of fitness (measured as lifetime reproductive success as stated above), in order to measure the adaptive potential in its strictest sense (i.e. standardised measure of additive genetic variance of fitness). To do so, we used a zero-inflated Poisson model with independent parameters on the latent traits corresponding to the zero-inflated binomial and Poisson processes. The zero-inflation is mainly due to low survival to reproduction [53]. A model including genetic and environmental correlations between the two latent traits did not yield significant correlations.

Computation of the quantitative genetic parameters From the output of the animal models, quantitative genetic parameters (population mean, phenotypic variance V_P, additive genetic variance V_A and heritability h^2) were computed using the QGglmm R package [14], using the relevant error distribution for each trait and integrating over the posterior distribution of each parameter. The variances from all random effects were used in the computation of the total phenotypic variance. Note also that the values computed for V_P and thus h^2 account for the variance explained by fixed effects [54]. Where relevant for the trait (i.e. not for non-Gaussian traits nor for laying date, for which the population mean is too arbitrary for CV_A to be meaningful), the coefficients of variation of the additive genetic variance (CV_A) were computed as the square-root of the additive genetic variance divided by the population mean, multiplied by 100. For fitness (lifetime reproductive success), we also estimated the evolvability I_A (V_A divided by the squared population mean) as a standardised measure of adaptive potential [55, 56].

Estimation of selection Selection gradients were computed for each trait, centred to a mean of 0 and scaled to a variance of 1. We used a Poisson generalised linear model (as implemented in the glm R function of the stats package) to account for the skewed and discrete nature of fitness and computed the gradients and their standard-errors based on [13].

Simulation analysis In order to assess the ability of our sampling design (i.e., sample size, along with the pedigree data structure) to estimate *small, but substantial* heritabilities, we performed a simulation analysis. We used the pedigree of the Tiritiri Matangi hihi population to simulate traits according to five different scenarios: *(i)* the trait is individual-based (all fledglings are measured once) and normally distributed, typical of e.g. tarsus length; *(ii)* the trait is breeding-based (only breeding females are measured, but with 3 measures *per* female) and normally distributed, typical of e.g. laying date; *(iii)* the trait is individual-based (all individuals are measured once) and binary (typical of e.g. recruitment status); *(iv)* the trait is breeding-based and binary (i.e. composed of zeros and ones, with 3 measures per female), typical of e.g. hatching success and *(v)* a zero-inflated Poisson trait with latent mean and variance comparable to our fitness trait. Because heritability below an arbitrary threshold of 0.1 would be regarded as small (close to 0.1) to inconsequential (close to 0), we decided to use 0.1 as the simulated heritability. For the binary trait, parameters were set so that the heritability on the observed data scale (rather than the latent scale or liability) was 0.1. Since scenario *(v)* was about detecting additive genetic variance of fitness and the heritability is not the best measure for this trait [56], we used an evolvability (or additive genetic variance of relative fitness) of 0.01 for the Poisson process, which corresponds to a heritability of 0.04. Since the meaning of evolvability for binomial traits is less obvious, we kept a heritability of 0.1 for this (part of the) trait in scenario *(v)*, which would correspond to an evolvability of 0.03. As a result of using the pedigree from our study, the sample sizes of each simulated scenario closely followed ours (see simulation code hosted in Dryad). Additionally, in order to follow the structure of our fitted models and account for the precision lost by adding random effects, we simulated the effects of social sire, dam and year of birth for scenario *(i)*, *(iii)* & *(v)* and of individual ID (permanent environment), mate ID and year of breeding for scenarios *(ii)* & *(iv)*. All of the simulated effects were added as random effects in the fitted models. The variance of those random effects were set equal to 1, with a residual variance of 1.5 for the Gaussian trait and a "residual" variance of 1 (as it is fixed for MCMCglmm) for binary traits. Each scenario consisted of 100 replicates, fitted in MCMCglmm, for which we computed the posterior mode, median and 95% credible interval of the estimated heritability.

Data and Software availability

Phenotypic data, the pedigree and R code is available online on the UCL Discovery repository (DOI: 10.14324/ 000.ds.10065966). Hihi are of cultural significance to the indigenous people of New Zealand, the Māori, and are considered a taonga (treasured) species. For this reason, the genotypes, raw reads, assembled genome and RAD-seq reads for hihi will be made available by request on the recommendation of the iwi (tribe) that affiliates as kaitiaki (guardians) for hihi. To obtain up-to-date contact details for the iwi, please contact Dr Anna Santure: a.santure@auckland.ac.nz