



## Effect of extra-pair paternity on effective population size in a reintroduced population of the endangered hihi, and potential for behavioural management

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### Abstract

We collected genetic and behavioural data on hihi (*Notiomystis cincta*, an endangered New Zealand bird) after reintroduction to Mokoia Island to assess the effect of extrapair copulation on effective population size ( $N_e$ ), and investigate the potential for increasing  $N_e$  through behavioural management. DNA fingerprinting revealed that 46% of chicks ( $n = 188$ ) resulted from extra-pair paternity, and 82% of broods ( $n = 56$ ) had at least one extra-pair chick. Of the extra-pair young, 34% ( $n = 89$ ) were from unpaired males, and the remainder were from paired males. Variance in reproductive success (VRS) among individuals changed between years, and the relative variance among males and females depended on the sex ratio. VRS increased when measured over longer time scales, the variance in recruits being three times higher than the variance in the number of hatchlings. Extra-pair copulation increased VRS by 150% in 1 year and decreased it by 30% in another year, but this only caused a 4% decrease and 8% increase, respectively, to  $N_e/N$ . Although there is potential to manage VRS in this species through behavioural management, a more important factor is adult lifespan, which is the main correlate of lifetime reproductive success as well as the determinant of generation time. The high annual mortality rate in Mokoia hihi (females = 64%, males = 52%) has prevented the population from growing, so the key factors limiting  $N$  and  $N_e/N$  are the same.

### Introduction

The hihi (stitchbird, *Notiomystis cincta*) is an endemic New Zealand bird that was originally found throughout the North Island and several offshore islands. Following European colonisation, hihi were reduced to a single population on Little Barrier Island, in the northern part of their original range. The mainland extinction of hihi was probably caused by a combination of factors, including predation by introduced mammals (mainly ship rats, *Rattus rattus*), loss of habitat, and disease (Rasch et al. 1996). The conservation of many of New Zealand's threatened species depends on the establishment of viable populations

on islands with suitable habitat and no mammalian predators (Armstrong and McLean 1995). Since 1980, there have been translocations of hihi from Little Barrier Island to five other offshore islands in an attempt to establish new populations. Recent research has assessed factors affecting the viability of translocated populations on Kapiti Island (Castro et al. 1994), Mokoia Island (Armstrong et al. 1999; Armstrong and Perrott 2000; Castro et al. 2003) and Tiritiri Matangi Island (Armstrong and Ewen 2001), but has not yet taken genetic considerations into account.

One consequence of translocation is that there are often relatively few individuals released, resulting in loss of genetic diversity (Stockwell

et al. 1996; Ardern et al. 1997; Brookes et al. 1997; Fitzsimmons et al. 1997; Robichaux et al. 1997; Komdeur et al. 1998). Loss of genetic diversity can potentially result in inbreeding depression, where reproduction and/or survival rates are reduced. The loss of genetic variation will be exacerbated if there is extensive post-release mortality, meaning that the founder group is much smaller than the number of individuals released, and/or if the population fails to grow quickly, resulting in a chronically small population. In addition, the effective population size ( $N_e$ ) in terms of genetics may be much smaller than the number of individuals present at any time. Effective population size is lowered by skewed sex ratios, and differences in reproductive success among individuals (Newton 1989; Frankham 1995). Variance in reproductive success (VRS) can occur through non-random mating and/or better survival of offspring from some parents. High VRS, when few animals produce most of the young, lowers the effective population size, and over time reduces genetic variability. Consequently, assessing the potential for loss of genetic variation requires not only tracking the numbers of each sex over time, but also studying the population's mating system (Parker and Waite 1997).

Hihi have a complex and dynamic mating system. They typically form breeding pairs, but may also form polygynous, polyandrous or polygynandrous units (Castro et al. 1996). More importantly, there is a strong emphasis on extra-pair copulation. Fertile females are often harassed by multiple males attempting forced copulations, and may solicit copulations from extra-pair males (Castro et al. 1996). Analysis of the Tiritiri Matangi hihi population using minisatellite DNA fingerprinting showed that extra-pair paternity occurs, and may be achieved by unpaired males as well as paired males (Ewen et al. 1999). This means that extra-pair paternity could either reduce the VRS, by unpaired males achieving paternity, or increase this variation, by some males siring a disproportionate number of chicks. Addressing this issue requires a large sample size of broods and requires several years of data. Ewen et al.'s (1999) study involved 10 broods in two seasons and so is insufficient for this purpose.

In this paper, we assess paternity of hihi produced during the first 4 years after translocation to Mokoia Island, and use this to determine the VRS

among males in this population. Combining this information with data on VRS among females, we assess the effect of the mating system on  $N_e$ . We discuss the implications for conservation of hihi and other bird species with high rates of extra-pair copulation, and discuss the potential for using behavioural management to increase  $N_e$ .

## Materials and methods

### *Study site and translocation*

Mokoia (38°05'S; 176°17'E) is a small (135 ha) inland island on Lake Rotorua, New Zealand. Mokoia is covered by a mosaic of young regenerating vegetation (Perrott and Armstrong 2000), and is free of mammalian predators except for mice (*Mus* spp.), which are not known to prey on passerines in New Zealand although they prey on invertebrates and plants. Mokoia lacks natural tree cavities for hihi to nest in due to the young age of its vegetation. Nest boxes were therefore provided, greatly facilitating the observations made in this study. Forty hihi (20 males and 20 females) were translocated to Mokoia from Little Barrier Island in September 1994 (Armstrong et al. 1999).

### *Field methods*

All birds released on the island were individually colour banded and had blood samples taken. Young produced on the island were colour banded while in the nest, or shortly after fledging. Therefore, every adult bird in the population could always be individually identified. Hihi are sexually dimorphic, with males being larger and more colourful than females. Hihi are very tame and allow observers at close range. We used binoculars to read bands and, if the birds were not at close range, to identify the sex of individuals during our field observations. We surveyed the population twice each year from 1994 to 2001 to estimate survival, which was needed to calculate  $N_e$ . These surveys occurred at the start (late October) and the end (late March) of the breeding season, and involved walking the tracks on the island over a 2–3 day period recording all hihi seen. During the first four breeding seasons after translocation (1994/1995–1997/1998), we collected behavioural observations at all but two nests and took blood samples from all the young.

All nest boxes were monitored throughout the breeding season. During the pre-laying (6 days) and laying (1–5 days) periods combined, we watched nesting sites for a total of 724 h (average 14 h per clutch). These observations were used to determine which males were resident at the nest sites, and which males attempted to copulate with the female. Males were considered resident if they did territorial calls, guarded the female, and chased other birds within 50 m of the nest site as described in Castro et al. (1996). In most cases, these observations were supported by records of the same male(s) feeding chicks at the same sites after egg hatching. A male resident at any nest site was considered to be “paired” for that year, and other males were considered to be “unpaired”. We recorded the mating behaviour of the resident male and female at each nest site, and the identity and mating behaviour of all other visiting hihi. In 1995, we observed nest boxes during the laying of every egg to determine whether females were laying eggs in other females’ nests (nest parasitism). Egg laying was highly asynchronous in the other 3 years of the study, so it was very unlikely that nest parasitism could have occurred in those years. In addition, no unsuspected extra eggs were found in any of the nests during our daily checks.

We took blood samples from chicks while they were in the nest, and muscle samples from chicks that died before blood samples were taken. Blood samples were taken from chicks once they weighed >20 g (after day 10). The maximum amount of blood collected from any chick in our study was equivalent to 7.7% of the total blood volume, less than the recommended conservative value of 10% (Coles 1985). Blood was collected by venipuncture of the ulnar vein directly into 50  $\mu$ l capillary tubes. In order to recover dead chicks, nests were checked three times per day while the female was foraging. This was necessary because female hihi discard dead chicks soon after death. Muscle and blood samples were initially kept in liquid nitrogen and then stored permanently in the laboratory at  $-80^{\circ}\text{C}$ . Unhatched eggs were recovered and cracked to determine whether there were embryos in them, and embryos found were stored in the same way as other tissue samples. However, these embryos turned out to be too small or too decomposed to use for DNA fingerprinting.

#### *Minisatellite DNA profiling and assessment of parentage*

We analysed parentage for 56 of the 67 clutches laid on Mokoia during the 4 years of study. The 56 clutches involved 46 different families, a family consisting of the resident female, resident male(s) and all of the hatchlings at a nest site. We could not use 11 clutches (10 families) for the following reasons: (1) Dead chicks from two single-chick families were discarded by the female, and we were unable to find them. (2) Females from two clutches died during early incubation, and we were unable to extract DNA from the embryos. (3) The blood sample from one female (two clutches, one family) was lost due to liquid nitrogen evaporation. (4) Four females’ entire clutches were found smashed before the hatching date, and no embryos were found. (5) One chick was found as an independent fledgling, but his nest was never located.

The methods used for extraction, precipitation and re-suspension of DNA from tissue samples, Southern blotting, and for stripping membranes are described in Ewen et al. (1999). The only difference in our methods is that we used probes *per* (Shin et al. 1985) and pV47.2 (Longmire et al. 1990) in addition to Jeffreys 33.15 (Jeffreys et al. 1985). We also used different hybridisation temperatures and times, and different exposure times, for each probe. For example, hybridisation was carried out at  $68^{\circ}\text{C}$  for at least 4 h with probe *per*, at  $60^{\circ}\text{C}$  for at least 12 h with probe pV47.2, and at  $68^{\circ}\text{C}$  for at least 12 h with Jeffreys 33.15. Pre-hybridisation was carried out for 2 h at the same temperatures used for hybridisation. After hybridisation, membranes were washed twice with  $5 \times \text{SSC}/0.1\% \text{SDS}$  at the same temperature used for hybridisation. Once the membranes were dry, they were exposed on X-ray film at  $-80^{\circ}\text{C}$  with one intensifying screen. Membranes were exposed for 8–72 h with probe *per*, whereas they were exposed for 1–8 days with probes pV47.2 and Jeffreys 33.15. Each membrane was probed with *per* first, and then with at least one other probe (i.e., pV47.2 or Jeffreys 33.15), with the exception of membranes obtained from secondary gels (see below) which were only probed with *per*.

Our strategy for loading DNA onto gels depended on the number of broods produced by a family each year. For families with a single brood, we loaded DNA from the chicks in the centre of

the gel, loaded their social parents (i.e., the female and the male resident at the nest site) at either side, and loaded all remaining wells with DNA from potential extra-pair sires. For families with two broods, we loaded the social parents between the chicks belonging to each clutch, and loading the remaining wells with potential extra-pair sires. Extra-pair males observed at the nest site were loaded close to the chicks, and other males present on the island were loaded on the remaining wells if available. For 1995/1996, we were able to load most males on the island on each gel. However, the high operational sex ratios (up to 14 males to 1 female) in 1994/1995, 1996/1997 and 1997/1998 made this impossible, and males never observed at the nest site were initially excluded from gels. If we had doubts that the sires were included on these initial gels after scoring the DNA profiles, we loaded secondary gels including the chicks, social parents, and remaining extra-pair males. Excluding 10 clutches where we ran the chicks with every available male on the island (including secondary gels), on average, we ran each chick with eight males ( $SD = 2.5$ ; range 3–12). All gels had molecular weight markers in the outermost lanes to ensure even running within gels and to allow estimation of band sizes, and included one common individual as a genomic control (Millar et al. 1994). Bands were transposed onto acetates (Galbraith et al. 1991) and compared among individuals (Bruford et al. 1991; Lambert et al. 1994), but were only scored if clearly identifiable.

We scored bands in the 3–24 Kb range for probe *per*, and in the 2–6 Kb range for probes Jeffreys 33.15 and pV47.2. Combining all probes used, an average of 18 bands were scored per individual bird (range 9–30,  $SD = 5.3$ ,  $n = 49$  fingerprints). The average number of scorable bands for individual probes was 15.0 ( $SD = 4$ ,  $n = 12$ ) with *per*, 6.2 ( $SD = 3$ ,  $n = 12$ ) with pV47.2, and 4.5 ( $SD = 4$ ,  $n = 8$ ) with Jeffreys 33.15. We assigned parentage using novel bands (Westneat 1990, 1993) and band-sharing coefficients (Wetton et al. 1987; Bruford et al. 1991).

#### Statistical analyses

We examined genetic paternity as well as the putative paternity (based on resident males and assuming no extra-pair paternity). Reproductive success was analysed by looking at three recruit-

ment categories: the number of hatchlings, fledglings and recruits each male and female produced. We calculated the VRS for sires, putative fathers and unpaired males separately.

We used two-way ANOVA (SYSTAT 1992) to test for the effect of year (1994/1995, 1995/1996, 1996/1997, 1997/1998) and male pairing status (paired and unpaired) on the number of chicks sired per year (hatchlings). We considered that the individual male paternity in 1 year was independent from his paternity in subsequent years. We used variance ratio tests to determine whether extra-pair paternity caused a significant change in standardised VRS among males using genetic versus observation data. Likewise, we used variance ratio tests to compare the standardised VRS of males and females between years.

We calculated mean and variance in adult lifespan from annual survival probabilities. We estimated survival probabilities from survey data using the Cormack–Jolly–Seber model in program MARK (White and Burnham 1999). Birds were considered to enter the population when first observed as adults (at the start of their first breeding season or later), and were then recorded as observed or not observed in subsequent surveys. We considered 25 alternative models in which survival and resighting probabilities were explained by different combinations of factors, including sex, season (October–March or March–October) and time (each interval treated separately). We selected the model that best explained the data, using Akaike's Information Criterion ( $AIC_c$ ), and used that model to estimate survival probabilities for males and females.

We calculated  $N_e/N$  using the following equation described in Parker and Waite (1997), which followed Waite and Parker (1997) and Nunney (1993). Because sex ratios were different between the years, we calculated  $N_e/N$  for each year separately.

$$N_e/N = \frac{4r(1-r)T}{r[A_f(1+I_{Af}) + I_{bf}] + (1-r)[A_m(1+I_{Am}) + I_{bm}]}$$

where  $r$  is the proportion of males in the breeding population,  $T$ , the generation time, measured as  $M + A - 1$ , where  $M$  is the time to sexual maturation and  $A$ , the average of  $A_f$  and  $A_m$  (see below),  $A_f$  and  $A_m$  are the average lifespans for males and females, respectively,  $I_{Af}$  and  $I_{Am}$ , standardised

variances (variance/mean<sup>2</sup>) for female and male adult lifespans respectively,  $I_{bf}$  and  $I_{bm}$ , standardised variances (variance/mean<sup>2</sup>) for female and male reproductive success, respectively. We included unpaired males in our calculations of standardised VRS because they can father offspring as extra-pair males (Ewen et al. 1999).

#### *Ethical note*

Blood samples from chicks were taken under Massey University Animal Ethics Committee Approval 95/150.

## **Results**

The hihi breeding population consisted of 33 birds in 1994/1995, 24 birds in 1995/1996, 30 birds in 1996/1997, and 33 birds in 1997/1998. We observed 16 females in 1994, 13 in 1995, 12 in 1996, and 14 in 1997. The majority of birds nested as part of monogamous units, with a maximum of eight polyandrous units (two in 1995–1996, four in 1996–1997 and two in 1997–1998).

#### *Behavioural observations*

In addition to the resident male(s), from zero to nine extra-pair males were observed at each nest site during the fertile period of the resident female (mean = 3.6; SD = 2.0). All males present during this period attempted to copulate with the female, but few of these attempts resulted in successful copulations.

#### *Parentage analysis*

Band sharing among individuals was generally high (Blood: mother and chick =  $0.7069 \pm 0.1$ ,  $n = 144$ ; sire and chicks =  $0.6966 \pm 0.1$ ,  $n = 140$ ; non-father and chicks =  $0.5567 \pm 0.1$ ,  $n = 977$ ; Muscles: mother and chick =  $0.6112 \pm 0.2$ ,  $n = 49$ ; sire and chicks =  $0.6411 \pm 0.1$ ,  $n = 43$ ; non-father and chicks =  $0.4989 \pm 0.2$ ,  $n = 282$ ). We found that band sharing was lower for muscle samples than for blood samples and that these differences were significant ( $t$ -tests assuming unequal

variances: mother and chick  $t_{2,144,49} = 1.9949$ ,  $P = 0.0004$ ; sire and chicks  $t_{2,140,43} = 2.0003$ ,  $P = 0.0272$ ; non-father and chicks  $t_{2,977,282} = 1.9658$ ,  $P = 0.000$ ). However, bandsharing on Mokoia was similar to that among the original hihi translocated from Little Barrier Island (mean  $\pm$  SD,  $0.5 \pm 0.1$ ). In addition, the average band sharing calculated for mothers/chicks and for sires/chicks was significantly higher from that for non-sires/chicks (two sample  $t$ -test: mothers versus non-sires  $t_{144,997} = -12.69$ ,  $P < 0.0001$ ; sires versus non-sires  $t_{140,977} = -11.76$ ,  $P < 0.0001$ ).

We observed the following rules to ensure that paternity was assigned to each chick as conservatively as possible: (1) If more than one male had zero novel bands with a chick, and one of them was the male resident at the nest, we assumed the resident male was the sire only if his band sharing with the chick was similar to that between the mother and the chick. (2) If more than one male had zero novel bands with a chick, and the resident male was not one of them, we assumed the sire was the male with the highest band-sharing coefficient with the chick among those with zero novel bands. (3) When there was no combination of adult males and females that had zero novel bands with a chick, and all possible extra-pair sires were loaded with such a chick, we assigned the chick to the male with which it shared a single novel band, or in six cases, with two novel bands, in combination with the highest level of band sharing. Again, if the resident male was one of these males, we assumed he was the sire if his band-sharing coefficient was similar to that between the mother and the chick.

We were able to assign paternity to 188 of 194 chicks analysed (Table 1, Figure 1). We could not resolve parentage for six chicks, four of which shared all bands with the mother, and two of which did not produce clear band profiles. Of the 188 chicks for which paternity was assigned, 150 were completely compatible with their assigned parents, 32 had one unattributable band present in their DNA profiles and six had two unattributable bands in their DNA profiles. We assumed these bands resulted from mutations because the mutation rate calculated from the bands we scored ( $44/12835 = 3.4 \times 10^{-3}$ ) lay within the range of mutation rates reported for other species (Jeffreys et al. 1985; Burke and Bruford 1987; Burke et al. 1989; Westneat 1990; Ardern et al. 1997; Verboven and Mateman 1997).

Table 1. Paternity assignment for hihi chicks on Mokoia Island based on DNA profiling

Year	No. of chicks profiled	No. of chicks sired by		Unassigned (%)
		Social father (%)	Extra-pair (%)	
1994	42	18 (43)	23 (55)	1
1995	48	27 (56)	20 (42)	1
1996	52	30 (58)	20 (38.5)	2
1997	52	24 (46)	26 (50)	2
Total	194	99 (51)	89 (46)	6 (3)

### Paternity and reproductive success

During each of the four breeding seasons, every male ( $n=44$ ) on the island attempted to breed (was observed seeking copulations). Extra-pair paternity was common, 46% of chicks being the result of extra-pair copulations for all years combined (Table 1, range 38.5–55%). There were only 11 clutches (18%) where the chicks were all sired by the resident male(s) (four of these clutches were polyandrous nests, and both males sired chicks). The other 50 clutches (82%) had at least one extra-pair chick, and in 15 (30%) of these clutches, the resident male sired none of the chicks. Up to three different males sired chicks at a single nest (mean = 2.0).

Unpaired males achieved paternity as extra-pair males at various nest sites, and paired males achieved paternity both with their partners and with females at other nest sites (Figure 2). The overall reproductive success of paired males was significantly higher than that of unpaired males (two-way ANOVA,  $F = 8.055$ ;  $df = 1$ ;  $P = 0.006$ , Table 2). Reproductive success did not vary among years ( $F = 0.380$ ;  $df = 3$ ;  $P = 0.768$ ), and there was no interaction between the effect of year and pairing status ( $F = 0.469$ ;  $df = 2$ ;  $P = 0.629$ ). On average, paired males achieved more paternity at their nests (mean  $\pm$  SD;  $2.3 \pm 1.71$  chicks) than did extra-pair males ( $1.35 \pm 1.38$ ) (Paired  $t$ -test:  $t_{2,43} = 2.02$ ,  $P = 0.003$ ). Unpaired males sired an average of 1.6 young, which was not significantly different from the number of extra-pair young sired by paired males (two sample  $t$ -test;  $t_{47,18,2} = 1.998$ ,  $P = 0.541$ , Table 2). Only 5% of paired males ( $n = 43$ ) did not sire any chicks at all, in comparison to 45% ( $n = 20$ ) of unpaired males. Unpaired males had significantly higher (variance ratio test:  $F_{1,18,42} = 2.8$ ,  $P < 0.005$ ) standardised

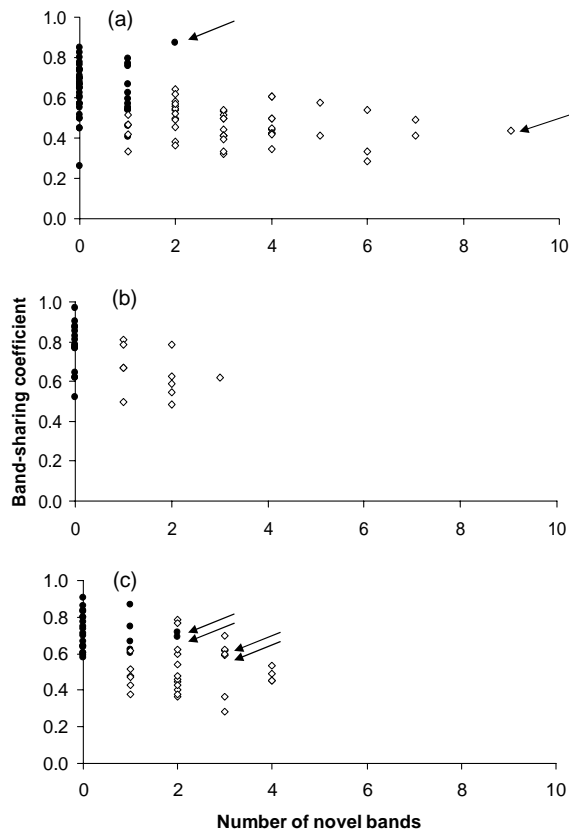


Figure 1. Relationship between the band-sharing coefficient and the number of novel bands for each extra-pair nestling. ● = most likely sire; ◇ = putative father. (a) Obtained using three minisatellite probes (*per*, pV47.2 and Jeffrey's 33.15). (b) Obtained using minisatellite probe *per*. (c) Obtained using two minisatellite probes (*per* and pV47.2 or *per* and Jeffrey's 33.15). Arrows point at the relationship between the band-sharing/novel band combination for extra-pair chicks with two novel bands and their sire (●) and putative father (◇).

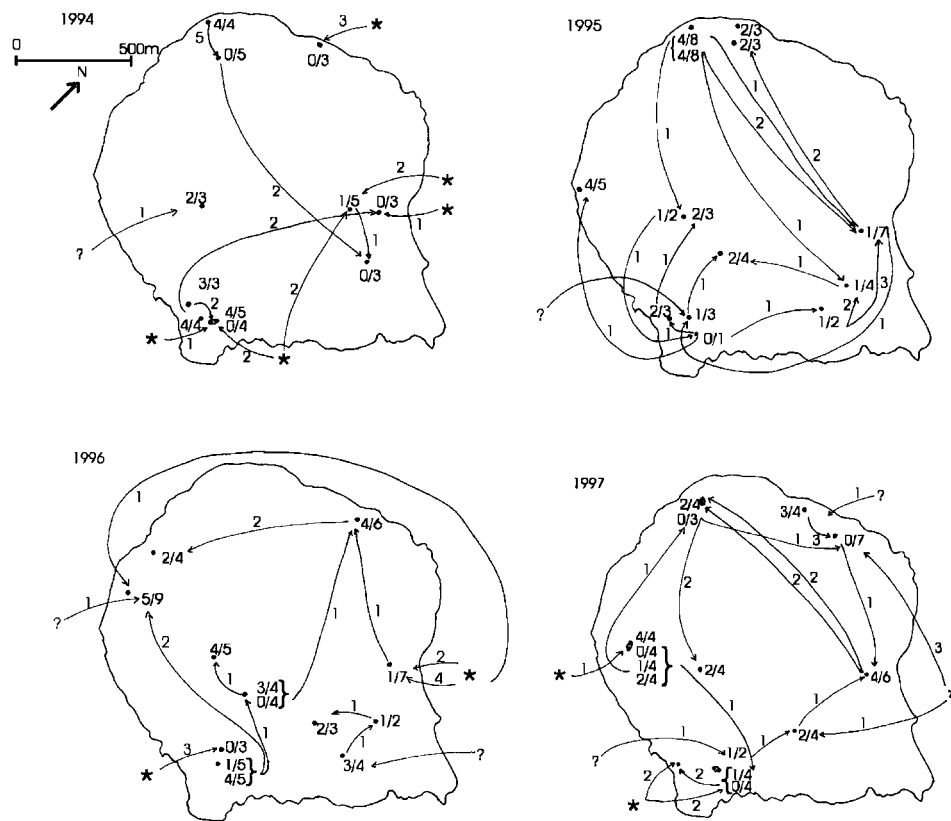


Figure 2. Mokoia Island map showing the nest sites and the paternity each male achieved each year at his nest (ratio numbers = Number of chicks sired over total number of chicks at the nest) or as EPCs (numbers over the arrow lines). Arrow points show where males sired EP chicks. Each asterisk outside the map represents one unpaired male. Question marks (?) indicate the paternity for a chick was not resolved.

VRS (variance divided by the mean squared, 1.22) when compared to paired males (0.44).

Most of the males on the island at any time were paired, and paired males were responsible for

Table 2. Number of paired and unpaired males and number of males achieving paternity (sires) for the 4 years of the study

Year	Mean chicks sired (SD)						Sires (%)
	Paired males			Unpaired males (%)			
	n	Nest	EPP	Nest + EPP	n	EPP	
1994	8	2.3 (2.3)	1.5 (1.7)	3.8 (3.7)	9	1.2 (1.7)	10 (59)
1995	11	2.5 (2.0)	1.8 (1.5)	4.3 (2.5)	0	–	11 (100)
1996	11	2.7 (1.4)	0.8 (1.0)	3.5 (1.9)	7	1.6 (1.9)	14 (78)
1997	14 <sup>a</sup>	2.0 (1.3)	1.3 (1.4)	3.3 (1.9)	3	3 (1.7)	17 (94)
Totals	43	2.3 (1.7)	1.4 (1.4)	3.7 (2.4)	19	1.6 (1.6)	52 (81)

<sup>a</sup> We were unable to determine paternity at the nest for one male because his partner died early during incubation and we were unable to use the embryos within the eggs for DNA fingerprinting. He has been excluded from calculations.

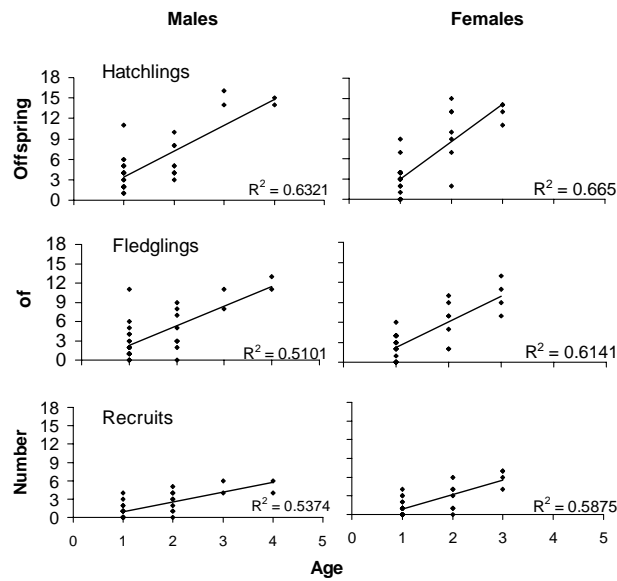


Figure 3. Correlation between the total number of hatchlings, fledglings and recruits sired (left) or mothered (right) by individual hihis and the number of breeding seasons the birds were alive from 1994 to 1997.

76% (142) of all chicks assigned. Only 13 males never paired, and only three males shifted from one condition to another. One of these males shifted from paired to being unpaired, and was suffering from aspergillosis at the time of the shift. The other two males shifted from unpaired to paired.

Distance between nest sites on Mokoia ranged from 150 to 1690 m (Figure 2). Paired males achieved extra-pair paternity over this whole range of distances. Of the 30 instances of extra-pair paternity, the nest was within 500 m of the male's territory in 12 instances, between 600 and 1000 m in 14 instances, and greater than 1500 m in four instances (mean =  $635 \pm 374$ ). Paired males achieved some of their extra-pair paternity at times when their own females were fertile, travelling up to 1323 m (mean =  $678 \pm 352$  m,  $n = 16$ ).

Despite some males siring more chicks than others in a year, the main factor affecting lifetime reproductive success of males was the number of years they lived (Figure 3). With the exception of 1994, when only 59% ( $n = 10$ ) of males sired chicks, most males sired chicks: 100% ( $n = 11$ ) in 1995, 78% ( $n = 14$ ) in 1996, and 94% ( $n = 17$ ) in 1997 (Table 2).

Although most males sired hatchlings, only 71% (135) of these chicks fledged and only 32%

(61) survived to become part of the breeding population themselves (Figure 4). Of the 34 (77%) males that achieved paternity, 22 (50%) produced offspring that subsequently produced progeny. Of the 20 males translocated from Little Barrier Island, only seven (35%) produced offspring that bred.

#### Maternity and reproductive success

All 36 females that survived to the breeding season attempted to breed at least 1 year, except one bird that had a broken leg. Most (85%) of the females attempting to breed produced at least one hatchling, and 25 (69%) produced offspring that bred. Of the 20 females translocated from Little Barrier Island, 10 produced offspring that bred (50%). The

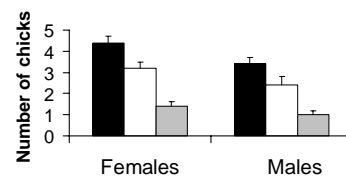


Figure 4. Number of hatchlings, fledglings and recruits produced per male and female surviving to breeding age from 1994-1997. Bars show means  $\pm$  standard errors.

Table 3. Proportion of males in the breeding population ( $r$ ) and standardised VRS of females ( $I_{bf}$ ) and males ( $I_{bm}$ ) in the 1994–1997 breeding seasons

Year	$r$	$I_{bf}$			$I_{bm}$ (genetic)			$I_{bm}$ (putative)		
		Hatchlings	Fledglings	Recruits	Hatchlings	Fledglings	Recruits	Hatchlings	Fledglings	Recruits
1994	0.52	0.56	0.98	1.55	1.55	2.70	2.95	1.51	2.22	3.27
1995	0.46	0.79	0.69	1.30	0.34	0.51	0.59	0.20	0.19	0.18
1996	0.60	0.35	0.53	1.31	0.66	0.85	1.91	0.94	1.32	2.63
1997	0.58	0.39	0.93	2.22	0.76	0.89	2.83	0.77	1.13	2.06

Reproductive success was calculated as the number of hatchlings, fledglings and recruits produced by each male and female, and male reproductive success calculated based on genetic versus putative parentage.

reproductive success of females, like that of males, was correlated with the number of years breeding (Figure 3).

#### Variance in reproductive success

The standardised VRS varied greatly between years regardless of how reproductive success was measured (Table 3). Overall, mothers had lower standardised VRS than sires, although in 1995–1996 mothers' standardised variance was significantly higher (Table 3) than that of putative fathers' (variance ratio test = 3.95,  $F_{13,11,0.05} = 2.79$ ). For all parent categories, the standardised variance increased from hatchling to recruits (Table 3). We considered that the variance observed at the hatching stage reflects mating success (i.e., non-random mating), while variance at the recruitment stage reflects chick quality (i.e., survival of the fittest). Standardised VRS of males was highest in 1994 and lowest in 1995. Comparing calculations based on genetic and putative parentage showed that extra-pair copulation increased VRS (hatchlings) by about 150% in 1995 and decreased it by about 30% in 1996. Extra-pair copulation had little effect on other years, with the apparent effect depending on how reproductive success was measured (Table 3).

#### Effective population size

Adult survival rates were estimated using the model  $\phi_{g+s}$ ,  $P_{g+s}$ , which was the best model for explaining the resighting data. This means that both survival and resighting probability varied between sexes (g) and seasons (s), and that the

seasonal changes in survival probabilities differed for the two sexes (i.e., there was an interaction between these factors). Under this model, the estimated annual survival probability ( $\hat{\phi}$ ) was 0.361 for adult females and 0.483 for adult males. Life expectancy is approximated by  $1/(-\ln \hat{\phi})$  based on the exponential distribution, giving mean adult lifespans of 0.98 years for females ( $A_f$ ) and 1.37 years for males ( $A_m$ ). Assuming there is no heterogeneity in survival probability among individual males and females, the standardised variance is 1.00 for both female lifespan ( $I_{Af}$ ) and male lifespan ( $I_{Am}$ ). Hihi are sexually mature by the first breeding season after they fledge, so  $M = 1$  and  $T = 1.18$ . These values were used for each of the 4 years in Parker and Waite's (1997) equation. The proportion of males in the breeding population ( $r$ ) was 0.52, 0.46, 0.60 and 0.58 for the 4 years.

Combining the above values with the standardised VRS from Table 3, we obtained the estimates of  $N_e/N$  presented (Table 4). Following the changes in standardised variance, estimates of  $N_e/N$  also depended on the timescale over which productivity was measured, averaging 0.39 if productivity was measured in terms of hatchlings and 0.29 if it was measured in terms of recruits. The effects of extra-pair copulation on VRS had relatively minor effects on our estimates of  $N_e/N$ . The 150% increase in variation in 1995 translated to a 4% decrease in  $N_e/N$ , and the 30% decrease in variation in 1996 translated to an 8% increase in  $N_e/N$  (Table 4). However, the effects in terms of effective population size are significant. For instance, in a hihi population of 30 individuals, the effective population size may be as small as 10 for hatchlings, or eight for recruits.

Table 4. Estimates of  $N_e/N$  based on genetic analysis and putative parentage for three measures of reproductive success (number of hatchlings, fledglings, and recruits into next year's breeding population) for the Mokoia hihi population

Year	$N$	$N_e/N$					
		Hatchlings		Fledglings		Recruits	
		Genetic	Putative	Genetic	Putative	Genetic	Putative
1994	33	0.35	0.35	0.28	0.30	0.26	0.25
1995	24	0.40	0.41	0.39	0.42	0.35	0.38
1996	30	0.41	0.39	0.38	0.36	0.30	0.27
1997	33	0.40	0.40	0.36	0.35	0.24	0.26
Average	30	0.39	0.39	0.35	0.36	0.29	0.29

## Discussion

The Mokoia hihi population had high levels of both extra-pair paternity (46% of chicks sired by extra-pair males) and mixed paternity (82% of nests having chicks sired by more than one male). These results are also similar to those obtained by Ewen et al. (1999) for hihi on Tiritiri Matangi Island (extra-pair paternity 35%, mixed paternity 80%). Such high values are usually found only in polygynandrous species (Mulder et al. 1994; Brisson et al. 1998).

Although most adult males (77%) and most adult females (86%) produced hatchlings over the 4 years studied, only 50% of males and 69% of females produced offspring that bred (recruits). Of the 40 birds (20 male, 20 female) translocated to Mokoia, 33 survived to the breeding season and only 17 (7 males, 10 females) produced offspring that bred, reducing the size of the founder group to 43%. The low percentage of males successfully breeding in the first year may be an artefact of leg injuries caused by the colour bands initially placed on the birds (Armstrong et al. 1999). All translocated males suffered leg injuries of varying degree, although only eight males were crippled by the time the breeding season started. Only one of the 16 females that survived to the breeding season had such an injury, and she never nested during the two breeding seasons she was alive. In addition to having a large percentage of males not breeding, one male sired 11 of the 42 chicks produced in 1994. These two factors explain the high standardised variance in male reproductive success for all recruitment categories in the first year in comparison to other years.

Males had the lowest standardised genetic VRS (across recruitment categories) in 1995. This is not surprising because the sex ratio in 1995 was biased towards females, and all males were paired and achieved paternity. In 1996 and 1997 when the sex ratio was biased towards males, polyandry was more common. The average number of chicks sired per male was nearly double in 1995 when compared to 1996 and 1997.

Female standardised variances in some instances appear to have been affected by factors other than those affecting males. The female standardised variance in hatchlings produced was very high in 1995 compared to other years. In that year, three females produced 26 of the 49 hatchlings, whereas the remaining 23 chicks were produced by seven females and three females produced none. The female standardised variance in fledglings produced was highest in 1994 and 1997. Mortality of the entire clutches due to disease in those years (unpublished data) is probably responsible for such discrepancies. Castro et al. (2003) found that female age and supplementary food significantly affected recruitment in hihi. This suggests that annual VRS may also be affected by the proportion of females of various ages in the population, and on food supply.

Disease may be playing an important role in determining the recruitment on Mokoia. The main cause of hihi mortality after fledging was found to be aspergillosis, a fungal infection responsible for up to 67% of all deaths (31 out of 65 birds) from 1995 to 1997 (Alley et al. 1999). Predation and other diseases were responsible for 9% of deaths, whereas the cause of death was unknown in the remaining 28% of cases (Alley et al. 1999). In the

absence of the disease, the proportion of surviving young may have differed from what we found. Furthermore, susceptibility to the infection could have a genetic component, reducing the chances of becoming ill on certain genetic lines more than others. This hypothesis is supported by a higher variance in the number of recruits produced in comparison to the number of hatchlings produced. Furthermore, a family tree constructed from the genetic data used in this paper shows that the birds surviving to 1997 are descendants from very few family lines, and the number of family lines represented became smaller each year.

Parker and Waite (1997) calculated  $N_e/N$  from data on extra-pair paternity in four bird species. In two socially monogamous species, purple martins (*Progne subis*) and blue tits (*Parus caeruleus*), some males had a disproportionate number of extrapair fertilisations, resulting in an increase in  $I_{bm}$ . This resulted in an average decrease of about 8% in both  $N_e/N$  and  $N_e$  in purple martins, and 2% in blue tits. In two cooperatively breeding species, stripe-backed wrens (*Campylorhynchus nuchalis*) and Arabian babblers (*Turdoides squamiceps*), genetic analysis showed that subordinate males that had not been considered part of the breeding population were siring offspring. Adding subordinate males to the breeding population resulted in an increased  $I_{bm}$ , and corresponding decrease in  $N_e/N$ , because most subordinate males sired few or no offspring. However, adding these males increased  $N_e$ , and meant that extra-pair fertilisation increased  $N_e$  in these species, by 5% in stripe-backed wrens and 15% in Arabian babblers.

We found similar effects to those described in Parker and Waite (1997) in that extra-pair copulation allowed unpaired males to sire offspring. Because we had already considered these males to be part of the breeding population, these extra-pair fertilisations reduced  $I_{bm}$  and increased both  $N_e/N$  and  $N_e$ . This effect was clearest in the 1996 breeding season, when there was a strongly male-biased sex ratio. We also found that extra-pair paternity could allow some paired males to sire numerous offspring, increasing  $I_{bm}$  and reducing  $N_e/N$ . This effect was clearest in the 1995 breeding season, when there was low overall variance in male reproductive success due to a female-biased sex ratio. These effects cancelled each other out in the other 2 years, resulting in no clear effect on  $I_{bm}$ . Although extra-pair copulation had a

strong effect on variance in male reproductive success in two of the 4 years, this translated into relatively minor changes to the estimated  $N_e/N$  (4% decrease on 1995 and 8% increase in 1996). It therefore appears that  $N_e/N$  is not highly sensitive to changes in  $I_{bm}$  in hihi, similar to purple martins and blue tits.

Anthony and Blumstein (2000) have outlined multiple ways in which behavioural traits can affect  $N_e$ , and suggest that behavioural research can therefore play a major role in conservation of populations. This is part of a major groundswell of opinion in recent years suggesting there should be a greater emphasis on behavioural research in conservation biology (e.g., Curio 1996; Clemmons and Buchholz 1997; Caro 1998, 1999; Sutherland 1998; Gosling and Sutherland 2000). Hihi should be a prime species for applying behavioural research, not only because their mating system has the potential to affect  $N_e$  but also because we have management techniques available to manipulate their behaviour. We have successfully manipulated hihi mating on Mokoia Island by experimentally changing the distribution and abundance of food and nest boxes (unpublished data). We can therefore use these techniques to manipulate VRS to some extent in hihi populations. However, it seems likely that behavioural management would have a minor effect on the  $N_e/N$  values on Mokoia, and would therefore do little to prevent the loss of heterozygosity in this population.

Manipulating the parameters in Parker and Waite's (1997) equation suggests that the most important factor limiting  $N_e/N$  for Mokoia Island hihi has been adult lifespan. Hihi can potentially live for many years (banded birds have been known to live for 5, 7, and 11 years, pers. obs.), but the high annual mortality rate on Mokoia (64% for females, 52% for males) means that a hihi reaching breeding age has had a further life expectancy of only 1.2 years. If these mortality rates were halved, the average  $N_e/N$  value would increase by 21% and the  $N_e/N$  value based on recruits would increase by 36%. Equalisation of male and female mortality might also bring sex ratios closer to 1:1, but changes to sex ratios would have only a minor effect on  $N_e/N$ . With the current parameters, changing sex ratios to exactly 1:1 would increase  $N_e/N$  by only 3%.

Halving the mortality rates on Mokoia would bring mortality rates in line with those estimated

for the reintroduced hihi population on Tiritiri Matangi Island, so this exercise is not an overestimation. Reducing mortality rates would have a more profound effect than increasing  $N_e/N$  in that it would also increase  $N$ . The high mortality rate on Mokoia prevented the population from growing, and unfortunately this problem could not be immediately redressed by management (Armstrong and Perrott 2000). This resulted in tenuous viability even with intensive food and nest site management (Armstrong et al. 2002), and the remaining birds were relocated to another island in 2002. However, if some genetic lines are more resistant to aspergillosis, it is possible that over time a population would develop that could survive and expand on Mokoia. If the population had been able to grow, but constrained to having a small population size, it is possible, that behavioural management could have played a role in preventing loss of heterozygosity. However, the Mokoia hihi population's viability was limited by a basic demographic factor related to poor habitat quality. Although genetic and behavioural considerations were of theoretical interest, they had little relevance to the conservation of this particular population.

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