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A field technique for determining the sex of North Island kokako (*Callaeas cinerea wilsoni*)

IAN FLUX
Science and Research Division, Department of Conservation, P.O. Box 10-420, Wellington, New Zealand
iflux@doc.govt.nz
JOHN INNES
Landcare Research Ltd, Private Bag 3127, Hamilton, New Zealand

Abstract North Island kokako (*Callaeas cinerea wilsoni*) appear sexually monomorphic. Females are, on average, slightly smaller than males in most body measurements. Mean tarsus length was significantly smaller among females at all sites and can be used to predict sex of unknown birds with up to 86% accuracy. A simple discriminant function, using tarsus and wing chord measurements, was derived which increased sex resolution to over 90% at some sites. This is sufficient accuracy to provide a useful field technique for kokako research and conservation management. Best discriminant functions for different sites are presented and their geographical limitations are discussed.

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INTRODUCTION

The North Island kokako (*Callaeas cinerea wilsoni*) is a member of an endemic family, the Callaeatidae or New Zealand wattlebirds. The kokako is the only wattlebird remaining on the New Zealand mainland; populations inhabit mainly small and disjunct areas of lowland forest in the central and northern North Island (Innes & Flux 1999). The species is considered to be threatened (Collar *et al.* 1994). A large, but variable, proportion of each population consists of pairs that are territorial throughout the year.

There are only 2 other species in the Callaeatidae, the extinct huia (*Heteralocha acutirostris*) and the saddleback (*Philesturnus carunculatus*). The huia was renowned for the obvious sexual dimorphism of its bill (Turbott 1967; Moorhouse 1996). Male and female North Island saddlebacks are visually similar but can be sexed reliably using tarsus length alone (Jenkins & Veitch 1991). Kokako, it seemed, exhibited no visible sexual dimorphism. Establishment of new populations, by translocation to predator-managed sites throughout the historic range of the species, is recommended in the Kokako Recovery Plan (Innes & Flux 1999). To establish new populations demands some ability to identify sex of birds to be translocated. Similarly, research into aspects of behaviour, ecology, and breeding of kokako would be aided greatly by a simple sexing technique.

Standard measurements (Gurr 1947) were recorded from kokako captured between 1990 and 1999. Sex of 111 adults was determined concurrently by field observation at Mapara Wildlife Management Reserve, King Country and at Rotoehu Forest, Bay of Plenty, during 7 years of intensive field research and management (Innes *et al.* 1999). Similar information was recorded from a small number of known-sex kokako handled in the Hunua Ranges (Auckland), Great Barrier Island, Moki Forest (Taranaki), and the Waimana Valley (Te Urewera). Here we present a summary of the most useful measurements for sex determination at the study sites and discuss the applicability of these results to determining sex at other sites.

STUDY SITES AND METHODS

Study sites

The 2 main study sites —Rotoehu Forest (440 ha), near Rotorua and Mapara Wildlife Management Reserve (1400 ha), near Te Kuiti — are both in the central North Island. The vegetation at both sites is lowland, mixed broadleaf forest with scattered podocarps. At Mapara the forest is isolated by surrounding pasture and young plantation forestry, where extensive control of introduced mammalian predators was undertaken between 1989 and 1997.

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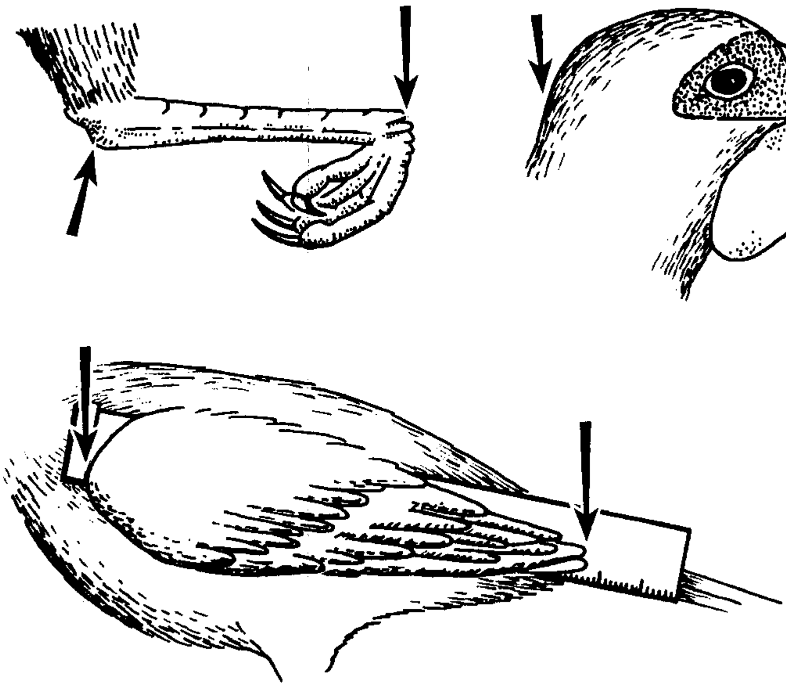

Fig. 1 Standard measurements recorded for North Island kokako (*Callaeas cinerea wilsoni*); tarsus length, head+bill, and wing chord.

The Rotoehu study area is a part of a larger (2900 ha) forest area. Intensity of pest control effort between 1994 and 1997 was similar to that at Mapara. The intensive pest control greatly increased the breeding success of kokako and new pairs became established at both sites (Innes *et al.* 1999). In consequence we found and monitored successful nests, which facilitated the field determination of sex.

Behaviour

From 1991 to 1997 a sample of marked pairs at both Mapara and Rotoehu was checked weekly from early November until all breeding activity ceased between February and May. If breeding took place the respective role of each partner during courtship, copulation, incubation, and chick rearing was recorded whenever possible.

Cloacal examination

During 1994 and 1995 the cloacal morphology of each kokako captured at both main study sites was externally examined and described.

DNA sampling and analysis

During 1997 and 1998 blood samples were collected from a sub-sample of the birds handled at Mapara. About 75 ml of blood from each bird was collected from the brachial vein and stored in an Eppindorf tube containing *c.* 0.75 ml of "Queen's Lysis Buffer" (10mM Tris-HCl; 10mM NaCl; 10mM EDTA; 1% N-lauroylsarcosine; pH 7.5). Each tube was labelled with the bird's band number and sent to Dr M. Double (Australian National University), who determined sex for these samples by analysis for the sex chromosome linked CHD-W gene (Griffiths *et al.* 1998).

Measurements

Paired and single adult kokako occupied territories throughout the year. Pre-recorded song was played to lure kokako into mist-nets rigged in the forest canopy within previously-identified territories. All kokako captured were fitted with a unique combination of leg-bands, 1 metal and up to 3 of coloured plastic. The kokako were measured and some were fitted with radio transmitters to assist in the later location of nests.

All measurements were made on live birds. Vernier callipers calibrated to ± 0.1 mm were used for all linear measurements except for wing chord which was measured to ± 1mm using a butt-ended ruler. Body weight was measured to ± 1g using a 300 g Pesola® spring balance. Tarsi were measured with the leg forward against the bird's body and the foot flexed back (Fig.1: Gurr 1947). With the leg in this position we measured the lower leg from the intra-articular notch at the proximal end to the articulation with the toes. Head+bill was measured as the greatest distance from bill tip to rear of skull (Fig. 1). Wing chord was measured with the wing against the bird's body in the resting position. The wing was straightened against the ruler then released before the measurement was made (Fig. 1).

Birds caught at Rotoehu from 1989 until 1990 were weighed and wattle length, wattle breadth, head width, lengths of head+bill, wing chord, tarsus, mid-toe, and mid-toe-with-claw, were measured. Only weight, wattle dimensions, mid-toe with claw, and tarsus were measured in 1991-1994. One observer (Paul Jansen) measured nearly all of the birds. Birds caught at Mapara from 1990 to 1995 were weighed and tarsus length was measured. From 1996 until 1999, head+bill and wing chord lengths were also measured. All birds at Mapara were measured by IF.

Birds from various other sites throughout their current range were also measured by IF. Weight and length of wing chord, head+bill, and tarsus were recorded for these birds. Adults were re-measured if recaptured to assess whether any of these body dimensions changed over time and to provide an indication of the repeatability of measurements. Statistical analyses were done using SPSS Base 8.0 (SPSS Inc. 1998).

RESULTS

Behaviour

Observations of individually colour-marked breeding birds confirmed that each member of a pair performed different roles. During pre-breeding courtship 1 member of each pair consistently fed the other (very occasionally the reverse was observed) and was the usual instigator of "archangel" displays (Lovegrove 1980) where the bird raised its wings and moved rapidly in the tree close to its mate, often with some vegetation in its bill. Both Jenkins (1976) and Lovegrove (1980) reported that very similar behaviours in the North Island saddleback (*Philesturnus carunculatus rufusater*) were performed mainly by the male. For the following observations, sex determination was based on the prediction that the more active bird in the 2 behaviours, as exhibited by kokako, was also the male.

The female generally built the nest alone, though in a few pairs the male contributed small amounts of building material. Copulation observed between members of several pairs took place in a tree with the male perched on the female's back in the manner usual for passerines. Only females incubated and they also did all brooding of young nestlings. Males fed females on the nest at intervals of 10 - 85 minutes throughout the incubation and early nestling periods.

Our assignment of sex to colour-marked kokako was determined ultimately by incubation role as this was easily and reliably recorded. Using this marker, we determined the sex of 78 kokako at Mapara, 33 kokako at Rotoehu, and 19 birds from other sites. If the eggs of a given female were fertile, we assumed her mate to be male.

Cloacal examination

In some passerines the area around the cloaca becomes protuberant in males as sperm storage increases before breeding (Wolfson 1952). Cloacal morphology varied considerably between individual kokako, but at neither site did such variation appear to be correlated with the sex of the birds as determined from other criteria. A known female had a very protuberant cloaca. To avoid any possibility of disturbing nesting kokako or influencing nest outcomes, few kokako were caught during the nesting period so we cannot say whether the external cloaca changed near the onset of breeding.

Genetic sex marker

Chromodomain-helicase-DNA-binding W-linked gene (CHD-W) is a sex-linked gene found on the W chromosone of most birds (Griffiths *et al.* 1998). A 2nd, slightly different, version of the gene is found on the Z chromosone. PCR-primers are used to amplify a region of the gene including the coding sequence. Differences in size between the non-coding region of CHD-W and CHD-Z lead to a difference in the size of PCR products which can be detected using gel electrophoresis. Extractions from females (ZW) thus result in 2 product bands on a gel and those from males (ZZ) only yield 1 product band.

All 15 blood samples from Mapara gave clear, independent, indications of sex by this method. Samples from all supposed females exhibited 2 product bands and all males 1 band which corroborated field evidence of sex.

Morphometrics

In total, 130 individual kokako were measured during this study. Of these, 34 known-sex kokako at Mapara and 18 at Rotoehu were measured more than once. For most of the analysis, mean sizes were calculated for birds with multiple records. However, repeat measures gave us an opportunity to assess the repeatability of measurements and to look for any changes in size over time.

The results of kokako remeasured at least once, after periods of 1 - 6 years since they were first measured as an adult, are summarised in Table 1. Any trends in measurements with time were determined by plotting repeat measures and looking for well-supported regression lines. We then analysed repeat measurements amongst known-age individuals. The sample size of birds of known sex and age was small and no clear trends were apparent (Table 1).

Measurements of mid-toe-and-claw varied by up to 14% (mean 5.9%) between measurements of the same individual, presumably because it is difficult to fully straighten the toes. Wattles are small soft-tissue structures and measurements varied similarly, particularly wattle length for which repeat measurements differed by a mean of 10.1%.

The mean differences for weight at Mapara and Rotoehu were substantial (3.1% and 5.4%, respectively) but were influenced by a few birds whose weight fluctuated remarkably. One Rotoehu female increased 22% from 211 to 257g in 1 year. Such changes probably resulted from variation in the contents of the digestive tract but also could reflect real changes in condition resulting from differences in food availability between years, or in other environmental vari-

Table 1 Repeat measurements of known-sex North Island kokako (*Callaeas cinerea wilsoni*). No trend was apparent in any dimension.

Site	Measurement	Mean	No. of repeats	Mean difference	Mean difference as % of sample mean
Mapara	Weight (g)	219	34	7.18	3.1
	Tarsus (mm)	66	26	0.80	1.2
	Head+bill (mm)	59	10	0.93	1.6
	Wing chord (mm)	153	10	2.20	1.4
Rotoehu	Weight (g)	232	18	12.72	5.4
	Tarsus (mm)	67	15	0.99	1.4
	Wattle length (mm)	15.7	17	1.58	10.1
	Wattle depth (mm)	14.3	17	0.8	5.6
	Mid-toe+claw (mm)	42.2	14	2.51	5.9

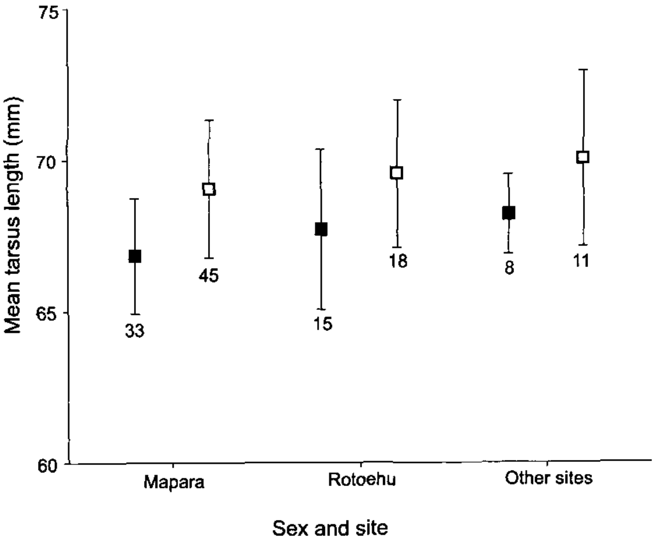

Fig. 2 Mean length of tarsus of known-sex North Island kokako (*Callaeas cinerea wilsoni*) from Mapara, Rotoehu, and other sites. Bars indicate 2 SD about mean. ?, females, ?, males.

ables. No trend in weight over time was apparent from the data. Most kokako were captured in the spring (August to November), so we could not detect possible seasonal changes in kokako condition.

No significant trend in size over time was detected for any linear measurement, so we assumed that differences were attributable to measurement error. Lack of a trend suggests that kokako do not grow significantly after they become territorial adults at 10 months to 3 years of age. Only in mean tarsus length at Mapara did an increase between measurements approach significance; the dimensions increased in 17 individuals as against a decrease in only 8 and 1 that was constant. There may have been a small increase in the length of this section over time but any such increase, if real, was masked by the measurement error. Despite such differences, measurements of the tarsus, head+bill, and wing chord were highly repeatable and so appeared to be the most useful measurements for further analysis.

Differences in measurement means were tested at a stringent ($P < 0.001$) level to determine which would be most diagnostic in sex determination. We then analysed differences between regional populations at a similar level to determine whether regional differences in size distributions masked any sexual differences. At all sites measurements of males tended to be larger than those of females (Fig. 2). Means of measurements from males were significantly larger than females ($P < 0.001$) for all Mapara birds where sample sizes were large (tarsus and weight samples represented about 75% of the then-current adult population). Tarsus lengths were also significantly ($P < 0.001$) greater in males than females at Rotoehu and at other sites. Only a few birds were measured at Rotoehu and other sites and differences between the means of their wing chord and head+bill measurements were not significant (Table 2).

Males from the 2 main study sites showed no significant differences in any measurement at the stringent ($P < 0.001$) level adopted. Surprisingly, however, the means for males at the combination of other sites were significantly larger than for males at Mapara for weight and wing chord and were close to being significantly larger for tarsus ($P = 0.007$). Females from Rotoehu were significantly larger than Mapara females for weight only. As with the males, females from the combined "other" sites were larger than those at Mapara in tarsus and nearly significantly different in weight ($P = 0.003$) and wing chord ($P = 0.002$).

The standardized canonical discriminant function coefficients (Table 2) show the relative contribution of each of the measurements in the determination of sex of an unknown bird at these sites. The larger the coefficient, the greater is its usefulness in sex-determination. Tarsus length was the most important measurement at all sites, followed by either head+bill, or wing chord, then weight (Table 2).

Best discriminant functions

Using tarsus, wing chord, head+bill, and weight, in a step-wise manner, we derived the best dis-

Table 2 Mean (± SD) measurements (linear, mm; weight, g) and values of Standard Canonical Discriminant Function Coefficient (D) for sex determination of North Island kokako (*Callaeas cinerea wilsoni*) by site. Higher values show greater utility in sex determination. ***, significant difference at $P < 0.001$ between means for males and females; M or R, differs at $P < 0.001$ from the Mapara (M) or Rotoehu (R) mean for this sex.

		Males		Females		
Site	Measure	*n*	Mean ± SD	*n*	Mean ± SD	D
Mapara	Tarsus	45	68.1 ± 2.3	33	63.7 ± 1.9 ***	.635
	Weight	46	229 ± 13.1	32	210 ± 12.3 ***	.087
	Head+bill	31	60.2 ± 1.4	23	58.1 ± 1.2 ***	.356
	Wing chord	31	157 ± 5.0	23	150 ± 6.3 ***	.150
Rotoehu	Tarsus	18	69.0 ± 2.4	15	65.4 ± 2.7 ***	.572
	Weight	17	235 ± 9.2	14	231 ± 14.0 M	.013
	Head+bill	9	60 ± 0.9	8	57.8 ±1.7	.508
	Wing chord	6	159 ± 3.3	9	150 ± 3.1	.557
Other	Tarsus	11	70.1 ± 2.9	8	66.4 ± 1.2 *** M	.865
	Weight	12	245 ± 19.3 M	9	224 ± 8.3	.164
	Head+bill	7	61.1 ± 1.2	6	58.4 ± 2.2	.636
	Wing chord	7	166 ± 5.8 M	6	159 ± 3.3 R	.293
All Sites	Tarsus	74	68.6 ± 2.5	56	64.5 ± 2.3 ***	.677
	Weight	75	233 ± 14.5	55	218 ± 16.8 ***	.064
Total	Head+bill	47	60.3 ± 1.3	37	58.1 ± 1.5 ***	.495
	Wing chord	48	158 ± 6.1	38	151 ± 6.3 ***	.120

criminant function for sex-determination at each site and for North Island kokako in general, based on these sites. For all, weight and head+bill were closely correlated with other measures, and so added little or no discriminatory ability. Tarsus measurements alone best separated the sexes at Mapara, but at Rotoehu and other sites the addition of wing measurements greatly increased the ability to discriminate. Different equations were most efficient at assigning sex at the different sites (t, tarsus length; w, wing chord). At Mapara, 86.1% of all birds (90.9% of females, 82.2% of males) were sexed correctly by the equation $t_{\text{males}} \geq 65.9\text{mm} > t_{\text{females}}$. The probability of a correct diagnosis was > 0.9 when t_{males} was > 68.1 and $t_{\text{females}} < 63.6$. At Rotoehu, 94.7% of all birds (88.9% of females, 100% of males) were sexed correctly by the equation $0.387t + 0.107w - 42.464 = x$, where $x \geq 0$ = male and $x < 0$ = female. The probability of a correct diagnosis was > 0.9 when x was > 0.97 for males and $x < -0.98$ for females. At other sites 92.3% of all birds (100% of females, 85.7% of males) were sexed correctly by the equation $0.387t + 0.124w - 46.066 = x$, where $x \geq 0$ = male and $x < 0$ = female. The probability of a correct diagnosis was > 0.9 when x was > 0.93 for males and $x < -0.92$ for females. For the combined data from all sites 82.6% of all birds (84.2% of females, 81.3% of males) were sexed correctly by the equation $0.397t + 0.038w - 32.424 = x$, where $x \geq 0$ = male and $x \geq 1.19$ = female. The probability of a correct diagnosis was > 0.9 when $x \geq 1.19$ for males and $x \leq -1.19$ for females.

DISCUSSION

Tarsus length was measured easily in the field and was the most consistently repeatable measurement on any particular bird; therefore we consider it to be the key measurement for use in determining the sex of kokako in the field. Measurements of wing length aided discrimination but they may vary with time as a result of a bird's condition and its state of moult. During September to April, when we measured most birds, wing chord was also highly repeatable, but wing measurements may be affected by moult and feather wear.

Given all the caveats, we feel that the equations should prove to be reasonably robust in practice. The discriminant functions, and the probabilities of correct diagnosis, apply to the original, known-sex sample only. To sex any new individual by the methods outlined it must be assumed that the original sample means, for all measurements, were representative of their respective population means. The sampling described in this paper included ~10% of individual kokako alive at the time of the study and included individuals from throughout the geographic range of the species at that time. Thus, it is unlikely that any populations will contain individuals that differ markedly from the size ranges we recorded (Fig. 2). The sex of kokako can be computed quickly in the field using the discriminant functions and an electronic calculator, allowing managers and researchers to make more informed decisions on individuals to select for translocations or other purposes. Scores range from –3 to +3, but any scores near 0 should be treated as indeterminate. In all instances, the more extreme the score the greater is the probability of a correct determination. Nevertheless, when time is available and the result is critical we recommend that the sex of kokako be determined by genetic analysis of blood, or from the behaviour of birds at nests.

Our study confirmed the previously-assumed sex roles in kokako breeding pairs. Mutual feeding was observed throughout the year but appeared to increase in frequency before breeding; almost always the male fed the female. Males, too, were the usual instigators of "archangel" (raised-wing) courtship displays. Same-sex pairings were common in kokako (I. Flux, unpubl. data) and involved both courtship feeding and "archangel" displays so the safest technique to sex banded birds without capture was to locate and observe nests. Female kokako usually build the nest and only female kokako incubate and brood. Male birds feed the female and chicks, both on and off the nest, but their visits to the nest are invariably brief.

We found no useful characters for ageing mature kokako. Kokako fledge at about 35 days with short tails, dull-olive-brown-tinged plumage, and small pink to lilac wattles, but with their tarsi at close to adult length. The latest we could safely handle nestlings was ~15-25 days by which time the largest were already within adult size ranges. Male chicks had tarsi of up to 65.7?mm long and the tarsi of the largest female chick were 62.1?mm. The legs of 1 female caught aged 3 months had not increased by 2 years. Similarly, the tarsi of a male measured at 8 months had increased only by 0.2?mm by the following year. The rate of maturation of wattle characters appears to vary. The wattles of some kokako are large and blue within 3 months of fledging whilst others have remnants of pink on full-sized wattles at 9 months.

In the North Island saddleback, wattle size was thought to be sex- and age-related (Jenkins & Veitch 1991). As with Jenkins & Veitch (1991), we found wattles difficult to measure accurately. One-year-old kokako in breeding pairs could not be distinguished from older birds by wattle size and there was no evidence of sex-related differences in the wattles. Wattles of captive kokako can appear dull and shrunken when not breeding whereas those of an adult at the start of breeding appear bright blue and tumid. The high variability noted in repeat measurements of wattles may have reflected real changes resulting from seasonal effects or the bird's condition at the time of capture.

Care must be taken in determining the sex of kokako at sites for which fewer data are available. In this study significant differences were detected in mean measurements from kokako of the same sex from different populations. Geographic size variation can evolve over very few generations even in freely dispersing bird species (Baker & Moeed 1979). Such variation is highly likely in kokako as they favour closed-canopy forest and will rarely cross more than a 100?m gap of open country. Forest destruction in the central North Island, over the past 200 years has resulted in kokako populations that have been reproductively isolated for many generations. Small morphometric differences could, therefore, be expected as a result either of independent evolution over many generations at a particular site or of past population bottlenecks that altered morphometric ranges. It is interesting, though, that the significantly larger birds in this study ('other' sites in Fig. 2) were a sample of birds from remnant populations throughout the current range of kokako, not from a single population. They include birds from the limits of that range in, Te Urewera, the Hunua Ranges (Auckland), Great Barrier Island, and the Moki forest in Taranaki. We suggest that the size variation may not necessarily be a result of geographic isolation.

Territorial kokako are at least 10 months old. From that age, we could detect no significant growth in any of the structures we measured. At Mapara, however, we noted that the differences approached statistical significance, suggesting that there may be a very slow rate of continued growth in tarsus length during adult life. If real this growth might help explain another feature of the data in Fig. 2, where it can be seen that mean tarsus length was lowest at Mapara, which has been subject to the longest period of management, and highest in the unmanaged, "other" sites. Unmanaged sites suffer high rates of nest predation (Innes *et al.* 1999). Kokako are, however, long lived, so age distributions in unmanaged sites are skewed toward older individuals. Innes *et al.* (1999) demonstrated that control of pest mammals markedly boosted nesting success in managed areas. It follows then that younger birds must now dominate in managed populations, such as Mapara. Rotoehu, with 3 years of pest management between 1994 and 1997, would be expected to have a population whose age distribution was between these 2 extremes. Precisely that pattern was indicated by the tarsus size distribution. Another explanation might lie, however, in the selective pressures posed by higher pest numbers: could larger kokako be better able to survive in the presence of mammalian predators?

Morphometric differences have been reported between captive- and wild-reared birds of some species (Higgins 1999). It is unclear whether such differences are structural (e.g., bone size) or superficial (e.g., condition). Smith & Wiemeyer (1992) attributed the apparently larger size in captive-reared eastern screech owls (*Otus asio*) to there being less wear on wings, bill, and claws in captivity. Nevertheless, different diets could affect growth via differences in the availability of nutrients. In the absence of information on the effects of diet or environment, the discriminant functions derived in this study should be applied with a great deal of caution in determining the sex of captive-reared kokako.

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