

Inbreeding and Loss of Genetic Variation in a Reintroduced Population of Mauritius Kestrel

STEVEN R. EWING,*†‡** RUDI G. NAGER,* MALCOLM A. C. NICOLL,† AURELIEN AUMJAUD,‡§§
CARL G. JONES,‡§ AND LUKAS F. KELLER*††

*Department of Environmental and Evolutionary Biology, Graham Kerr Building, Glasgow University, Glasgow, G12 8QQ, United Kingdom

†Centre for Agri-Environmental Research, School of Agriculture, Policy and Development, University of Reading, Earley Gate, P.O. Box 237, Reading, RG6 6AR, United Kingdom

‡Mauritius Wildlife Foundation, Vacoas, Mauritius

§Durrell Wildlife Conservation Trust, Les Augre Manor, Trinity, Jersey, Channel Islands, United Kingdom

Abstract: *Many populations have recovered from severe bottlenecks either naturally or through intensive conservation management. In the past, however, few conservation programs have monitored the genetic health of recovering populations. We conducted a conservation genetic assessment of a small, reintroduced population of Mauritius Kestrel (*Falco punctatus*) to determine whether genetic deterioration has occurred since its reintroduction. We used pedigree analysis that partially accounted for individuals of unknown origin to document that (1) inbreeding occurred frequently (2.6% increase per generation; $N_{ei} = 18.9$), (2) 25% of breeding pairs were composed of either closely or moderately related individuals, (3) genetic diversity has been lost from the population (1.6% loss per generation; $N_{ev} = 32.1$) less rapidly than the corresponding increase in inbreeding, and (4) ignoring the contribution of unknown individuals to a pedigree will bias the metrics derived from that pedigree, ultimately obscuring the prevailing genetic dynamics. The rates of inbreeding and loss of genetic variation in the subpopulation of Mauritius Kestrel we examined were extreme and among the highest yet documented in a wild vertebrate population. Thus, genetic deterioration may affect this population's long-term viability. Remedial conservation strategies are needed to reduce the impact of inbreeding and loss of genetic variation in this species. We suggest that schemes to monitor genetic variation after reintroduction should be an integral component of endangered species recovery programs.*

Keywords: Bambous Mountains, conservation genetics, effective population size, endangered species, *Falco punctatus*, gene-drop analysis, genetic variation, inbreeding, Mauritius Kestrel, pedigree

Endogamia y Pérdida de Variación Genética en una Población Reintroducida de *Falco punctatus*

Resumen: *Muchas poblaciones se han recuperado de cuellos de botella severos ya sea naturalmente o por medio de manejo de conservación intensiva. Sin embargo, en el pasado pocos programas de conservación han monitoreado la salud genética de poblaciones en recuperación. Realizamos una evaluación genética de una población pequeña, reintroducida de *Falco punctatus* para determinar si ha ocurrido deterioro genético desde su reintroducción. Utilizamos análisis de pedigrí que dio cuenta parcial de individuos de origen desconocido para documentar que (1) la endogamia ocurrió frecuentemente (incremento de 2.6% por generación; $N_{ei} = 18.9$), (2) 25% de las parejas reproductivas estaban compuestas de individuos relacionados cercana o moderadamente, (3) la diversidad genética se ha perdido en la población (pérdida de 1.6% por generación; $N_{ev} = 32.1$) más rápidamente que el correspondiente incremento en la endogamia y (4) ignorar la contribución de individuos desconocidos al pedigrí sesgará las medidas derivadas de ese pedigrí, lo que a la postre obscurecerá*

** Current address: Royal Society for the Protection of Birds, Sandy, Bedfordshire, SG19 2DL, United Kingdom.

†† Current address: Zoological Museum, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland, email lukas.keller@zm.unz.ch

§§ This paper is dedicated to the memory of Aurelien Aumjaud, a brilliant and committed conservation biologist. Paper submitted April 20, 2007; revised manuscript accepted September 21, 2007.

la dinámica genética prevaleciente. Las tasas de endogamia y de pérdida de variación genética en la subpoblación de *F. punctatus* que examinamos fueron extremas y entre las más altas que se han documentado en una población silvestre de vertebrados. Por lo tanto, el deterioro genético puede afectar la viabilidad a largo plazo de esta población. Se requieren estrategias de conservación remediales para reducir el impacto de la endogamia y la pérdida de variación genética en esta especie. Sugerimos que los planes para monitorear la variación genética después de la reintroducción deben ser un componente integral de los programas de recuperación de especies en peligro.

Palabras Clave: análisis de baja de genes, especies en peligro, endogamia, *Falco punctatus*, genética de la conservación, montañas Bambous, pedigrí, tamaño poblacional efectivo, variación genética

Introduction

Bottlenecked populations are of substantial conservation concern because they are subject to stochastic genetic changes that can reduce their viability (Frankham et al. 2002). Their small size leads to the loss of genetic variation through random drift, which compromises their ability to respond adaptively to novel selection pressures (Franklin 1980; Frankham et al. 1999). Furthermore, inbreeding occurs, which can threaten population fitness through inbreeding depression (e.g., Keller & Waller 2002). To avoid these potentially deleterious repercussions, conservation programs aim to minimize inbreeding and the loss of genetic variation by maximizing the population's effective size N_e (Caballero & Toro 2000). In captive-breeding programs, factors that affect N_e (i.e., mating patterns, reproductive output, and generation times) can be manipulated to minimize inbreeding and reduce the loss of genetic variability. In the wild, in contrast, populations are less amenable to manipulative management and are subject to demographic conditions that may substantially accelerate loss of genetic variation (Frankham et al. 2002). Paradoxically, in spite of this greater propensity for rapid genetic deterioration, little is known about the fate of genetic variation in recovering wild populations beyond the reintroduction phase (but see Haig & Ballou 2002; Jamieson et al. 2003).

Two techniques allow genetic variation and the incidence of inbreeding to be monitored in wild populations. First, changes in absolute levels of genome-wide variation can be characterized by molecular markers. This method has been applied widely to conservation issues in wild populations, for example, to resolve the effects of demography on historical levels of genetic variation (Groombridge et al. 2000) and to assess the geographic partitioning of variation within and between subpopulations (Ciofi & Bruford 1999). The molecular approach, however, may not provide meaningful assessments of genetic relationship where populations have endured a recent bottleneck that has resulted in the loss of historical genetic variation. Second, relative changes in the amount and distribution of genetic variation can be evaluated with pedigree analysis, which simulates the pas-

sage of genes across generations (Haig & Ballou 2002). Pedigree analyses have been applied successfully to the conservation of domestic breeds (Toro et al. 2000) and in the management of small captive populations (Haig et al. 1990), but rarely has it been applied to wild populations (Haig & Ballou 2002). This restricted use is unfortunate because pedigree analysis derives more accurate estimates of some important conservation genetic metrics than those calculated from molecular markers (e.g., inbreeding coefficients, Slate et al. 2004) and because it allows a unique insight into the genetic-microstructure of populations (Haig & Ballou 2002).

An important obstacle in applying pedigree analysis to wild populations is the absence of complete pedigree information for wild populations (Haig & Ballou 2002). Individuals with unknown ancestry traditionally are assumed to be unrelated to all members of the pedigree (i.e., they are accorded founder status), but this can overestimate the magnitude of genetic variation in the founder generation and bias subsequent genetic metrics derived from these pedigrees (Lutaaya et al. 1999). A number of analysis techniques that can partially take into account individuals of unknown origin have been developed (van Raden 1992; Ballou & Lacy 1995; Marshall et al. 2002), but they are rarely used in the analysis of wild pedigrees. We used such techniques in the analysis of the pedigree of the reintroduced Mauritius Kestrel (*Falco punctatus*).

The Mauritius Kestrel is a small forest-dwelling falcon endemic to the island of Mauritius in the western Indian Ocean. In the early 1970s the wild population dropped to only 4 known individuals due to habitat loss and pesticide contamination (Jones et al. 1995). This bottleneck resulted in the loss of 55% of the ancestral populations' allelic diversity and 57% of its heterozygosity (Groombridge et al. 2000). Subsequently, a conservation management program of captive breeding and manipulation of the productivity of wild pairs was implemented (Jones et al. 1991). This program has resulted in an increase of the wild population, which now numbers between 500 and 800 individuals split between 2 principal populations in the Black River Gorges and the Bambous Mountain range (Jones et al. 2002). The Bambous Mountain population was established in 1987 with the release of a

small number of captive-bred and captive-reared kestrels (Jones et al. 1995). This population has been intensively monitored since its reestablishment, and this monitoring has yielded a detailed pedigree spanning 10 generations and 720 individuals.

Our goal was to determine whether there has been further genetic deterioration in the Bambous Mountain population since its reintroduction. The severe reduction of variation in molecular markers during the population bottleneck precludes the effective use of molecular techniques in this species. We used the comprehensive pedigree information and several techniques that partially account for the contribution of unknown individuals to derive estimates of inbreeding coefficients, genetic variation, and effective population size. Our results indicate that ignoring the contribution of unknown individuals to a pedigree will bias the metrics derived from that pedigree sufficiently to obscure the prevailing genetic dynamics.

Methods

Study Population

We monitored the Bambous Mountain Mauritius Kestrel population intensively in each breeding season between 1987 and 2003. Known nest sites were surveyed in late August and early September for the presence of resident pairs. In addition, frequent surveys were carried out in areas of suitable habitat to detect kestrels breeding in previously undocumented locations. This monitoring regime was estimated to have missed only 6 successful nesting attempts between 1987 and 1997 (Groombridge et al. 2001). Breeding adults at occupied sites were identified via a unique color-ring combination allocated to them at fledging. Occupied nest sites were visited regularly throughout the season to record date of first egg, clutch size, hatch date, brood size, and number of fledglings.

Pedigree Construction

Information from the captive-breeding program and from the monitoring of wild birds was used to construct a pedigree for the Bambous Mountain population. We identified 24 founders (i.e., individuals at the base of pedigree lineages whose parents were unknown and who thus were assumed unrelated) from the Black River Gorges subpopulation from which offspring were taken between 1981 and 1989 for the captive-breeding program. Although we identified 24 founders of the Bambous Mountain population, the species as a whole can be traced back to only 4 wild birds in 1974 (Jones et al. 1995). Thus, the 24 founders of the Bambous Mountain population are in fact related to unknown degrees. Because relationships in the early postbottleneck population were not documented,

however, we were forced to assume that the founders of the Bambous Mountain population were unrelated.

Wild-born offspring were assigned to parents on the basis of observations of marked adults attending the nest. We assumed a female to be the mother of offspring if she repeatedly exhibited defense and incubation behaviors, whereas we assumed a male to be the sire if he repeatedly fed the female and offspring. We have not been able to verify the accuracy of this parental assignment method. Given the low level of genetic variance of this species (Groombridge et al. 2000), it is unlikely that offspring could be confidently attributed to their parents through currently available molecular markers. Extra-pair fertilizations (EPF) are a potential source of error in wild pedigrees (i.e., Keller et al. 2002). Although we possessed no estimates specifically for the Mauritius Kestrel, EPFs in raptors are generally rare (reviewed in Mougeot 2004). Furthermore, even moderate rates of EPFs (10–30%) result in unbiased errors in genetic parameters derived from pedigrees (Keller et al. 2002). We therefore considered it unlikely that EPFs would lead to substantial inaccuracies in our estimates of genetic parameters.

Pedigree Analyses

Obtaining pedigree information for an entire wild population is difficult; therefore, such pedigrees often comprise several individuals whose pedigrees are unknown (referred to as unknown individuals hereafter). Traditionally, unknown individuals are assumed unrelated to all other founders in the pedigree. Nevertheless, if the parents of the unknown individuals are related to other members of the pedigree, this assumption overestimates the magnitude of genetic variation in the founder generation. To prevent unknown individuals from biasing estimates of genetic variation, we used 3 methods that can partially account for incomplete pedigree data: gene-drop analysis and the methods of van Raden (1992) and Marshall et al. (2002).

Monte Carlo simulations are applied in gene-drop analysis to mimic the transmission of founder alleles through a pedigree (MacCluer et al. 1986). At the beginning of Monte Carlo iterations, each individual in the founder generation is allocated 2 unique alleles, which are then randomly passed through the pedigree. At the end of a simulation, each descendant has been randomly assigned a genotype of 2 founder alleles. This process is repeated 10,000 times. Gene-drop analysis partially accommodates unknown individuals because it is possible to prevent the segregation of their alleles through the pedigree (Ballou & Lacy 1995). From the summed replicates, one then derives unbiased estimates of genetic variation for only that fraction of the pedigree that is descended from the true founder population (i.e., 24 kestrels from the Black River Gorges populations; Ballou & Lacy 1995). Where an individual derives a portion of its genome from a pedigree

lineage of an unknown individual, it is only that unknown proportion of the genome that is excluded from the simulation and subsequent calculations (Ballou & Lacy 1995).

Gene-drop simulations were carried out with the genetic management package Population Management 2000 (Pollak et al. 2002). We calculated 3 different metrics: the effective population size, founder genome equivalents, and inbreeding coefficients. We investigated temporal changes in the magnitude of each parameter and used the cohort of offspring fledged each year as the unit for which the genetic metrics were obtained. In addition, to illustrate how unknown individuals bias pedigree-based metrics, some of the above parameters were also derived while leaving unknown individuals in the gene-drop simulations.

Measures of Genetic Variation and Inbreeding

EFFECTIVE POPULATION SIZE

We considered 2 estimates of the effective population size, N_e : the variance effective size (N_{eV}) and the inbreeding effective size (N_{eI}). These are defined, respectively, as the size of an idealized population that would give rise to the same variance in allele frequencies and the same rate of inbreeding as in the population under study (Crow & Kimura 1970). They were calculated according to the following equations:

$$N_{eV} = 1/2 * (1 - (H_t/H_0)^{1/t}) \quad \text{and} \quad (1)$$

$$N_{eI} = 1/2 * (1 - (1 - F_{\text{mean}})^{1/t}) \quad (2),$$

where H_t and H_0 are the expected heterozygosities of the population at time t and of the founder population, respectively, F_{mean} is the mean inbreeding coefficient in the population at time t , and t is the average number of generations that separate the founder and study populations (Lacy 1995). We also calculated the effective size/census size ratio (N_e/N) for our study population. Because N_e is calculated by gene-drop based on the loss of genetic variation observed within the subset of the population descended from known founders, it is also necessary that N should only include that subset of the breeding population descended from known founders. To obtain N , we summed the number of genomes in the cohort of breeding adults in 2003 that were inherited from known founders (i.e., $N = 60$).

FOUNDER GENOME EQUIVALENTS

The founder genome equivalent (f_{ge}) is defined as the number of distinct founders that would give rise to the genetic variation of the study population with no random loss of founder alleles across descendant generations (Lacy 1989, 1995). It quantifies the cumulative loss of variation since the founder generation and was calculated as

$$f_{\text{ge}} = 1/2 \text{ mk}, \quad (3)$$

where mk is the average coefficient of kinship among individuals in the study population (Caballero & Toro 2000).

INBREEDING COEFFICIENTS

The magnitude of inbreeding was quantified with 3 methods of inbreeding coefficient calculation, 2 of which take into account unknown individuals. First, we calculated Wright's (1922) inbreeding coefficient (F), which is defined as the probability of inheriting 2 alleles at a genetic locus that are identical by descent. This coefficient was derived by permitting the genes of all individuals at the base of pedigree lineages (i.e., founders and unknown individuals) to segregate in the gene-drop analysis. Second, we estimated what we call the gene-drop inbreeding coefficient (F_{GD}), which is relative to a founder generation that excludes unknown individuals and partially accommodates incomplete pedigree data. It is defined as the probability of inheriting 2 alleles at a locus that are identical by descent, conditional on the ancestries of both alleles being traceable to specified founders (Ballou & Lacy 1995). Because gene-drop analysis only calculates inbreeding coefficients based on that fraction of an individual's genome inherited from the known founders, calculations of mean inbreeding that use this coefficient must be weighted by the known fraction of the genome.

The third method of inbreeding coefficient calculation is that of van Raden (1992), which also accommodates incomplete pedigree data. van Raden's algorithm calculates inbreeding coefficients for unknown individuals and their descendants by making assumptions about their genetic relationship with other members of the pedigree. In a closed population, unknown individuals are unlikely to be genetically different from those individuals whose complete pedigree is known. Consequently, the relationship coefficient (r) within a group of unknown individuals should be similar to that in a contemporary cohort of individuals whose pedigree was complete. van Raden's (1992) algorithm calculates individual inbreeding coefficients by replacing the unknown relatedness of unknown individuals with the average relatedness of the contemporary group of individuals whose pedigrees are complete.

van Raden's inbreeding coefficients (F_{VR}) were calculated with the program PEDIG (Boichard 2001). Parents of unknown individuals were grouped according to the birth date of their offspring. We assumed that unknown individuals recruit into the breeding population at 1 year of age and that each was breeding for the first time when initially recorded. In total, 7 unknown parent groups were constructed in the pedigree. The first 6 comprised the parents of unknown individuals fledged during years 1993–2003. The last was composed of the 24 founders, which we assumed were unrelated to each other. The

van Raden algorithm is computed iteratively (for details see van Raden 1992). Convergence was reached within 3 iterations. Standard errors for values of mean inbreeding were computed on the basis of the individuals in each cohort.

Prevalence of Close and Moderate Inbreeding

Marshall et al. (2002) outlined a method that estimates the incidence of close and moderate inbreeding in incomplete pedigrees and also permits an assessment of the specific combinations of relatives involved in inbreeding events. Close inbreeding is defined as mating between full-sibs or parents and offspring (i.e., $F = 0.25$), and moderate inbreeding is defined as mating between half-sibs, offspring and aunts/uncles, grandchildren and grandparents, or double first cousins ($F = 0.125$; Marshall et al. 2002). To calculate rates of inbreeding, close and moderate inbreeding events were identified. For each category, we also calculated the total number of mating events in which the known pedigree of a pair would allow the detection of that category of inbreeding. Close and moderate inbreeding was then represented as a proportion of the number of cases in which that particular type of inbreeding would have been detectable. Rates of inbreeding can be combined to give an estimate of the total prevalence of close and moderate inbreeding as

$$F_{\text{tot}} = 1 - \prod (1 - F_j). \quad (4)$$

The calculation assumes that individual rates of inbreeding, F_j , are small and independent of one another.

Results

The Bambous Mountain population grew markedly over the course of the study period, from 12 kestrels in 1987 to a minimum size of 154 kestrels in 2002 (Fig. 1). Nevertheless, as the population expanded, the number of offspring from at least one unknown parent increased (Fig. 2). In total, 58 offspring had unknown parentage, which is only a small fraction of the pedigree (8.1%, $n = 720$). Predictably, this increase in unknown offspring resulted in a concomitant decline in the completeness of the Bambous Mountain pedigree. Pedigree completeness decreased from 100% in 1992 to 78.2% in 2003 (Fig. 2).

The mean inbreeding coefficient differed between methods of inbreeding coefficient calculation. van Raden's inbreeding coefficient detected the highest degree of identity by descent ($F_{\text{VR}} = 0.092$, $\text{SE} = 0.007$), followed by the gene-drop coefficient ($F_{\text{GD}} = 0.085$, $\text{SE} = 0.008$) and Wright's coefficient ($F = 0.077$, $\text{SE} = 0.007$). Inbreeding coefficients increased steadily over time (Fig. 3), from $F = 0.02$ in 1987 to $F_{\text{GD}} = 0.173$ or $F_{\text{VR}} = 0.182$ in 2003. When pedigree data were complete

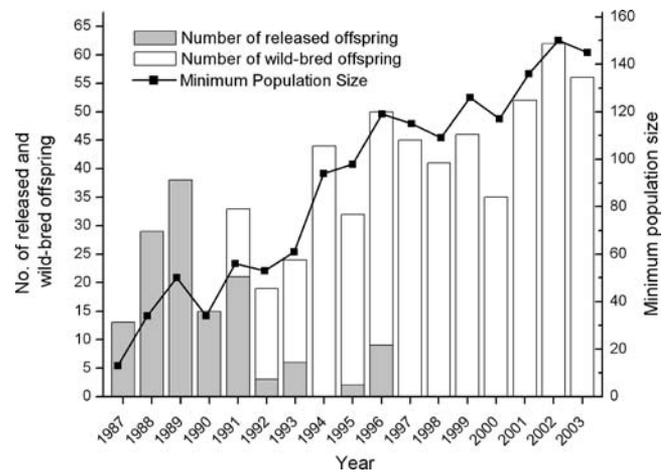


Figure 1. Annual changes in the minimum population size of the Bambous Mountain Mauritius Kestrel population and number of wild-bred and captive-reared offspring released in each year. Minimum population size is the postbreeding-season census size, including all known breeding adults and their fledged offspring.

(1987–1992), all 3 methods of calculation documented equivalent magnitudes of identity by descent (Fig. 3). Nevertheless, as pedigree completeness deteriorated, the estimates of mean inbreeding diverged and the degree of divergence was proportional to the relative contribution of unknown individuals to the pedigree.

Of 199 different breeding pairs, 19 consisted of closely related individuals, whereas 23 pairs were made up of moderately related individuals (Table 1). Thus, almost 1 in 4 breeding pairs (24.3%) were either closely or

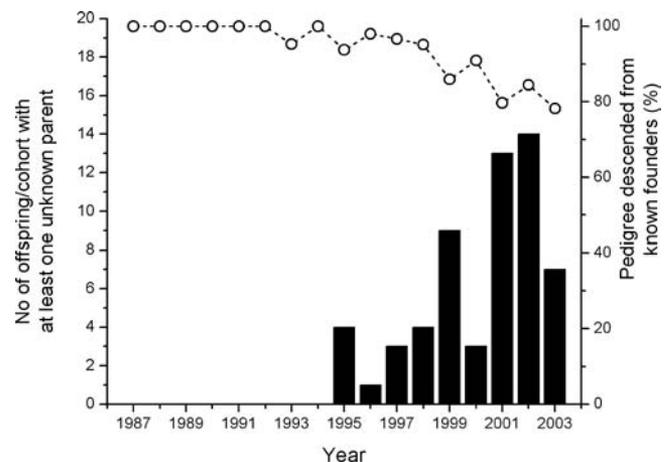


Figure 2. The number of Mauritius Kestrel offspring found each year with unknown parents and the resultant effects of this on pedigree completeness. The points marked by the open circles and dotted line represent annual changes in the percentage of the pedigree descended from known founders.

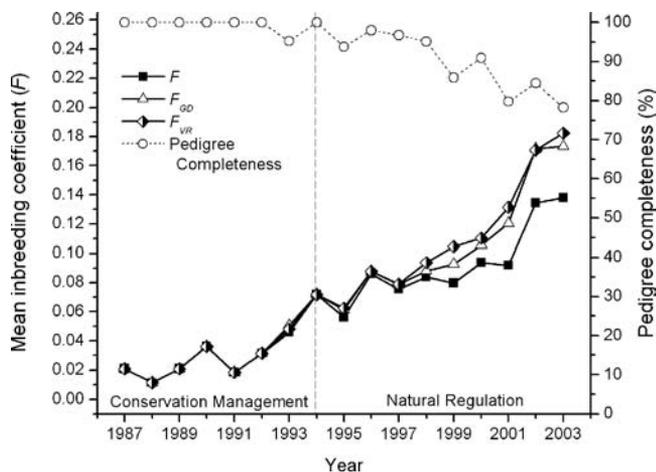


Figure 3. Inbreeding in the Bambous Mountain Mauritius Kestrel population since its reestablishment in 1987. The dotted vertical line is the point at which active conservation management of this population ceased.

moderately inbred. Within the class of close inbreeding, females paired up significantly more often with a brother (5.9%, $n = 152$) than with their father (1.7%, $n = 177$) ($\chi^2 = 3.87$, $df = 1$, $p < 0.05$), but sister-brother pairs were as frequent as mother-son pairs ($\chi^2 = 1.79$, $df = 1$, $p = 0.18$). Moderate inbreeding was mostly due to pairings of half-sibs and offspring with their uncles or aunts (20 out of 23 cases).

When unknown individuals were accommodated, founder genome equivalents (f_{ge}) gradually declined over time (Fig. 4; $r_s = -0.61$, $n = 17$, $p = 0.03$). In contrast, when unknown individuals were not accounted for, f_{ge} did not decline with time. Again, the degree to which the different values of f_{ge} diverged was a function of the relative contribution of unknown individuals to the pedigree. The value of f_{ge} fluctuated more during the period of conservation management than natural regulation because conservation practices sometimes inadvertently biased the genetic composition of offspring cohorts.

The variance effective population size (N_{ev}) was 32.1, whereas the inbreeding effective size (N_{ei}) was 18.9. These values equate to an average increase in the probability of identity by descent of 1.6 and 2.6% per generation, or 0.5 and 0.8% per annum, since the founder generation (per annum calculations assume a generation interval of 3.33 years; S.R.E., unpublished data). The N_e/N ratio of the Bambous Mountain population was either 0.315 or 0.535, depending on whether N_{ei} or N_{ev} was used.

Discussion

Conservation biologists emphasize the need to prevent loss of genetic variation and occurrence of inbreeding in endangered species because genetic variation is crucial

Table 1. Contribution of different combinations of relatives to rates of close and moderate inbreeding in the Mauritius Kestrel population in the Bambous Mountains.*

Male parent	Female parent	F	No. inbred/no. detectable	Frequency
Close inbreeding				
father	daughter	0.25	3/177	0.0169
son	mother	0.25	7/168	0.0417
full brother	full sister	0.25	9/152	0.0592
		Total	19	0.1137
Moderate inbreeding				
paternal half-brother	paternal half-sister	0.125	5/152	0.0329
maternal half-brother	maternal half-sister	0.125	3/152	0.0197
grandson	paternal grandmother	0.125	2/152	0.0132
grandson	maternal grandmother	0.125	0/155	0.0000
paternal/maternal grandfather	granddaughter	0.125	1/330	0.0030
full paternal uncle	full niece	0.125	4/140	0.0286
full maternal uncle	full niece	0.125	0/141	0.0000
full nephew	full paternal aunt	0.125	3/137	0.0219
full nephew	full maternal aunt	0.125	5/140	0.0357
double first cousin	double first cousin	0.125	0/123	0.0000
		Total	23	0.1454
			intragenerational	0.1081
			intergenerational	0.1507
			total	0.2426

*Total frequencies of inbreeding are calculated according to Eq. 4, which assumes that individual rates of inbreeding are small and independent of one another. It is not simply a case of summing the respective frequencies. Intergenerational inbreeding specifies mating between related individuals of different generations, whereas intragenerational inbreeding refers to mating between related individuals born in the same generation.

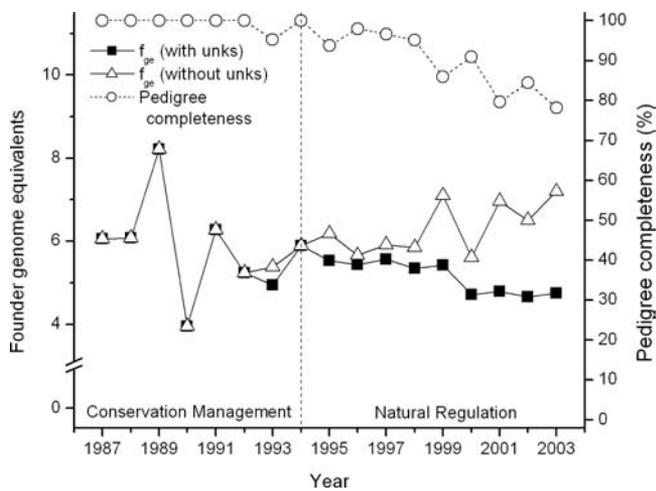


Figure 4. Temporal change in the number of founder genome equivalents (f_g) in the Bambous Mountain Mauritius Kestrel population between 1987 and 2003. This metric is calculated both accommodating (without) and ignoring (with) the contributions of unknown (unks) individuals. The decline in pedigree completeness is also illustrated. The dotted vertical line is the point at which active conservation management of this population ceased.

to their persistence. In this study we used novel methods of pedigree analysis that can partially accommodate the contributions of unknown individuals to gain insight into temporal changes of genetic variation and inbreeding in a small, but growing Mauritius Kestrel population. Our results showed that this population has accumulated inbreeding at a substantial rate since its reintroduction in 1987 and that approximately 25% of all matings were between closely or moderately related birds. Genetic diversity has been lost from the population, but this was less rapid than the rate of increase of inbreeding. When the contribution of unknown individuals to the pedigree was ignored, genetic metrics from the pedigree analyses were biased, thus obscuring the genetic dynamics of this population.

Impact of Unknown Individuals

Deriving accurate genetic parameters from pedigrees requires that all pedigree relationships be known (Haig & Ballou 2002). Nevertheless, where unknown individuals contribute to a pedigree, they invariably result in the underestimation of inbreeding and loss of genetic variation (Lutaaya et al. 1999). This was illustrated by the fact that both inbreeding coefficients that partially account for incomplete pedigree knowledge yielded 10.4–19.5% higher estimates than Wright's inbreeding coefficient. Using simulated pedigrees of varying completeness, Lutaaya

et al. (1999) investigated the extent to which Wright's and van Raden's inbreeding coefficients underestimate inbreeding. When 80% of dams in the pedigree were known, average inbreeding was underestimated by 60% and 30%, respectively, whereas 50% missing dams resulted in even higher underestimates (89% and 78%, respectively). For our study population pedigree completeness was <80% in only the final year of the study period, and over the whole study it was 91.9%. Less is known about the accuracy of the gene-drop methodology. Thus, the estimates of inbreeding and loss of genetic variation derived here should be considered minimum estimates for our study population.

The coefficients of inbreeding derived by Wright's (1922) procedure were lower than those of the other 2 methods because it does not account for unknown individuals. The difference we found between the gene-drop and van Raden's coefficients is likely to stem from the particular way in which the 2 techniques accommodate unknown individuals. Gene-drop coefficients account for unknown individuals by removing them and their subsequent lineages from pedigree analyses. Thus, if inbreeding occurs within the lineages derived from unknown individuals, this inbreeding is disregarded from calculations of mean inbreeding, and hence inbreeding is underestimated. In contrast, by assuming that unknown individuals are characterized by the same degree of inbreeding as contemporary cohorts of known individuals, van Raden's (1992) procedure accounts for inbreeding among descendants from unknown individuals. This explains the higher estimates of inbreeding derived by van Raden's (1992) technique. The degree to which the gene-drop and van Raden's coefficients differ, therefore, depends on the amount of inbreeding within lineages descended from unknown individuals.

We assumed that the known fraction of a pedigree is an unbiased representation of the entire pedigree. Nevertheless, certain characteristics can promote genetic differences between the known and unknown segments of a pedigree. In particular, immigration and poor sampling of geographical areas within a population can bias the unknown segment of a pedigree. The Bambous Mountain population is separated from its closest neighboring population by over 15 km of agricultural habitat, and no immigration events have been documented despite the existence of long-term ringing programs in both populations (C.G.J., unpublished data). Furthermore, all areas within the Bambous Mountains were sampled with similar effort. Therefore, in this kestrel population, unknown kestrels were almost certainly the product of undocumented breeding attempts of known parents. Consequently, the assumption of no genetic bias in the unknown segment of the pedigree appears justified. Nevertheless, these techniques may not be appropriate in populations where such biases are likely.

Inbreeding

Mean inbreeding was larger in the Bambous Mountain Mauritius Kestrel population (0.077) than in many other wild vertebrate populations, including those of other threatened species (e.g., Red-cockaded Woodpecker [*Picoides borealis*], Daniels & Walters 2000; Takahe [*Porphyrio hochstetteri*], Jamieson et al. 2003; North Island Robin [*Petroica longipes*], Jamieson et al. 2007). Reviewing the occurrence of close and moderate inbreeding in natural populations, Ralls et al. (1986) and Marshall et al. (2002) surmised that close inbreeding would generally be rare, accounting for only 0–6% of mating attempts, but that moderate inbreeding would be more common such that cumulative rates of close and moderate inbreeding may constitute between 5 and 15% of mating attempts. In our study population of Mauritius Kestrel, almost 1 in every 4 breeding attempts were between individuals related at the $F \geq 0.125$ level, which markedly exceeds the predicted range. Comparably high rates of close and moderate inbreeding have been demonstrated only in a small population of the Arabian oryx (*Oryx leucoryx*; Marshall et al. 2002) and a natural population of Splendid Fairy-Wrens (*Malurus splendens*; Ralls et al. 1986, but see Brooker et al. 1990 for additional details).

The high prevalence of inbreeding in the population of kestrels we studied can be explained by 3 characteristics of the population: few founders, a small effective population size and a lack of immigration. First, although 89 birds were released into the Bambous Mountain range during the reintroduction phase, many of these were related to one another, representing only 24 genetic founders. Second, the population has grown since the initial reintroductions, but it has nevertheless always contained relatively few individuals. In such small populations, genetic variation is depleted by genetic drift (Frankham et al. 2002). Finally, the Bambous Mountain population has remained closed to immigration from other populations; thus, there is no gene flow to replenish lost genetic variation (e.g., Keller et al. 2001).

The frequency of inbred matings varied between different combinations of relatives. Father–daughter breeding pairs were rarer than either son–mother or brother–sister combinations. This pattern is consistent with the predictions of Wheelwright et al. (2005), who suggest that there might be asymmetries in incest avoidance where the reliability of knowledge about kinship differs systematically among different categories of relatives, or where the costs associated with inbreeding are less severe for males than females. Adult male Mauritius Kestrels show a strong fidelity to their breeding territory, whereas fledgling males disperse to establish their own territories. Average natal dispersal distances of both sexes are short, however, averaging only 1–2 territory widths (Ewing et al., unpublished data). Females may avoid breeding with their fathers by ensuring that they do not pair with the res-

ident male on their natal territory, but the location of the eventual breeding territory of their brother is less predictable. This may explain why female kestrels paired more often with their brothers than with their fathers. Alternatively, brothers and sisters may pair more frequently with one another than with their parents because kestrels show long-term stable pair bonds and are therefore less likely to be available as breeding partners than same-age siblings.

Loss of Genetic Diversity

Small N_{EV} and temporal decline in f_{ge} imply that the Bambous Mountain population has lost genetic variation since its establishment. The magnitudes of N_{EI} and N_{EV} were different, such that inbreeding accumulated faster than variation was lost. The theoretical basis of this deviation is well known (Crow & Kimura 1970), but this finding has not been documented previously in any wild population. It occurs because N_{EI} changes proportionally to the average probability of identity by descent, which is influenced by the number of individuals in the grandparent generation, whereas N_{EV} characterizes allele frequency drift, which largely depends on the size of the progeny generation (Crow & Kimura 1970). Growing populations, such as the Bambous Mountain population, have progeny generations that are consistently larger than their corresponding grandparent generations, and this leads to larger N_{EV} than N_{EI} . There is a need for a greater appreciation that N_{EI} and N_{EV} may behave differently depending on the prevailing population dynamics, particularly in the field of conservation genetics where nonequilibrium dynamics are liable to be the norm.

Generalizations have been attempted to determine the range of values typically encompassed by the effective size/census size (N_e/N) ratio in wild populations. Frankham (1995) conducted a meta-analysis of the N_e/N ratio, and concluded that this ratio will commonly be of the magnitude 0.1, a value that is much smaller than recognized previously. Nevertheless, Waples (2002) suggested recently that the low estimate of N_e/N derived by Frankham (1995) is biased downward by a statistical bias in the treatment of fluctuating population size. The values of N_e/N we obtained ($N_{EI}/N = 0.35$ and $N_{EV}/N = 0.53$) are within the range (0.25–1) predicted by Nunney and Campbell (1993) and within the range typically observed in passerines (O'Connor et al. 2006).

Conservation Implications

Our results showed that the Bambous Mountain kestrel population has accumulated inbreeding and lost genetic variation. The rate of inbreeding (2.6%/generation) exceeded a 1%/generation inbreeding rate, a threshold beyond which natural selection typically cannot eliminate deleterious alleles from the gene pool (Franklin 1980;

Soulé 1980). Thus, natural selection may be unable to prevent the fixation of detrimental alleles in the Bambous Mountain population. In addition, due to the small N_{eV} , the population will continue to lose genetic variation rapidly, exacerbating its already compromised capacity to respond to novel environmental selection pressures. Given that this kestrel is already genetically impoverished and the retention of remnant variation is an important conservation priority, remedial strategies will be required to allay the accumulation of inbreeding and loss of genetic variation. The rarity of this species means that the potential conservation options are relatively limited, but one such strategy is the establishment of a low-intensity translocation scheme between existing populations. Artificial gene flow would ensure the preservation of genetic variation across the entire series of population fragments and that the incidence of inbreeding would be reduced. Furthermore, it would facilitate elimination of the drift load that can accumulate in subdivided populations by converting the drift load into segregating mutations (Glémin et al. 2003). Nevertheless, ultimately such a translocation scheme is a temporary measure. Ensuring the continued genetic viability of the Mauritius Kestrel will require a significant increase in the total N_e of the species, which will necessitate more broad-scale conservation measures such as widespread habitat regeneration.

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Literature Cited

- Ballou, J. D., and R. C. Lacy. 1995. Identifying genetically important individuals for management of genetic diversity in pedigreed populations. Pages 76–111 in T. J. Foote, editor. *Population management for survival and recovery: analytical methods and strategies in small population conservation*. Columbia University Press, New York.
- Boichard, D. 2001. PEDIG: a FORTRAN packed for pedigree analysis suited for large populations. *7th Congress on Genetics Applied to Livestock Production*, Montpellier, 19–23 August, paper 28–13. Available from http://www-sgqa.jouy.inra.fr/article.php3?id_article=110 (accessed 25 June, 2002).
- Brooker, M. G., I. Rowley, M. Adams, and P. R. Baverstock. 1990. Promiscuity: an inbreeding avoidance mechanism in a socially monogamous species. *Behavioral Ecology and Sociobiology* **26**:191–199.
- Caballero, A., and M. A. Toro. 2000. Interrelations between effective population size and other pedigree tools for the management of conserved populations. *Genetical Research* **75**:331–343.
- Ciofi, C., and M. W. Bruford. 1999. Genetic structure and gene flow among Komodo dragon populations inferred by microsatellite loci analysis. *Molecular Ecology* **8**:S17–S30.
- Crow, J. F., and M. Kimura. 1970. *Introduction to population genetic theory*. Harper & Row, New York.
- Daniels, S. J., and J. R. Walters. 2000. Inbreeding depression and its effects on the natal dispersal of Red-Cockaded Woodpeckers. *Condor* **102**:482–491.
- Frankham, R. 1995. Effective population size/adult population size ratios in wildlife: a review. *Genetical Research* **66**:95–107.
- Frankham, R., K. Lees, M. E. Montgomery, P. R. England, E. H. Lowe, and D. A. Briscoe. 1999. Do population size bottlenecks reduce evolutionary potential? *Animal Conservation* **2**:255–260.
- Frankham, R., J. D. Ballou, and D. A. Briscoe. 2002. *Introduction to conservation genetics*. Cambridge University Press, Cambridge, United Kingdom.
- Franklin, I. R. 1980. Evolutionary change in small populations. Pages 135–150 in M. E. Soulé and B. A. Wilcox, editors. *Conservation biology: an evolutionary-ecological perspective*. Sinauer Associates, Sunderland, Massachusetts.
- Glemin, S., J. Ronfort, and T. Bataillon. 2003. Patterns of inbreeding depression and the architecture of the load in subdivided populations. *Genetics* **165**:2193–2212.
- Groombridge, J. J., M. W. Bruford, C. G. Jones, and R. A. Nichols. 2001. Evaluating the severity of the population bottleneck in the Mauritius kestrel *Falco punctatus* from ringing records using MCMC estimation. *Journal of Animal Ecology* **70**:401–409.
- Groombridge, J. J., C. G. Jones, M. W. Bruford, and R. A. Nichols. 2000. Conservation biology—'ghost' alleles of the Mauritius kestrel. *Nature* **403**:616.
- Haig, S. M., and J. D. Ballou. 2002. Pedigree analyses in wild populations. Pages 388–405 in D. R. McCullough, editor. *Population viability analysis*. University of Chicago Press, Chicago.
- Haig, S. M., J. D. Ballou, and S. R. Derricks. 1990. Management options for preserving genetic diversity: reintroduction of Guam rails to the wild. *Conservation Biology* **4**:290–300.
- Jamieson, I. G., M. S. Roy, and M. Lettink. 2003. Sex-specific consequences of recent inbreeding in an ancestrally inbred population of New Zealand Takahē. *Conservation Biology* **17**:708–716.
- Jamieson, I. G., L. N. Tracy, D. Fletcher, and D. P. Armstrong. 2007. Moderate inbreeding depression in a reintroduced population of North Island robins. *Animal Conservation* **10**:95–102.
- Jones, C. G., J. J. Groombridge, and M. A. C. Nicoll. 2002. The genetic and population history of the Mauritius Kestrel *Falco punctatus*. Pages 297–304 in B. U. Meyburg, editor. *Raptors 2000*. WWGBP/Hancock House, Surrey, Canada.
- Jones, C. G., W. Heck, R. E. Lewis, Y. Mungroo, and T. Cade. 1991. A summary of the conservation management of the Mauritius Kestrel *Falco punctatus* 1973–1991. *Dodo* **27**:81–99.
- Jones, C. G., W. Heck, R. E. Lewis, Y. Mungroo, G. Slade, and T. Cade. 1995. The restoration of the Mauritius Kestrel *Falco punctatus* population. *Ibis* **137**:S173–S180.
- Keller, L. F., and D. M. Waller. 2002. Inbreeding effects in wild populations. *Trends in Ecology & Evolution* **17**:230–241.
- Keller, L. F., K. J. Jeffrey, P. Arcese, M. A. Beaumont, W. M. Hochachka, J. N. M. Smith, and M. W. Bruford. 2001. Immigration and the ephemerality of a natural population bottleneck: evidence from molecular markers. *Proceedings of the Royal Society of London Series B* **268**:1387–1394.
- Keller, L. F., P. R. Grant, B. R. Grant, and K. Petren. 2002. Environmental conditions affect the magnitude of inbreeding depression in survival of Darwin's finches. *Evolution* **56**:1229–1239.

- Lacy, R. C. 1989. Analysis of founder representation in pedigrees: founder equivalents and founder genome equivalents. *Zoo Biology* **8**:111-123.
- Lacy, R. C. 1995. Clarification of genetic terms and their use in the management of captive populations. *Zoo Biology* **14**:565-578.
- Lutaaya, E., I. Miztal, J. K. Bertrand, and J. W. Mabry. 1999. Inbreeding in populations with incomplete pedigrees. *Journal of Animal Breeding and Genetics* **116**:475-480.
- MacCluer, J. W., J. L. VandeBerg, B. Read, and O. A. Ryder. 1986. Pedigree analysis by computer simulation. *Zoo Biology* **5**:147-160.
- Marshall, T. C., D. W. Coltman, J. M. Pemberton, J. Slate, J. A. Spalton, F. E. Guinness, J. A. Smith, J. G. Pilkington, and T. H. Clutton-Brock. 2002. Estimating the prevalence of inbreeding from incomplete pedigrees. *Proceedings of the Royal Society of London Series B* **269**:1533-1539.
- Mougeot, F. 2004. Breeding density, cuckoldry risk and copulation behaviour during the fertile period in raptors: a comparative analysis. *Animal Behaviour* **67**:1067-1076.
- Nunney, L., and K. A. Campbell. 1993. Assessing minimum viable population size: demography meets population genetics. *Trends in Ecology & Evolution* **8**:234-239.
- O'Connor, K. D., A. B. Marr, P. Arcese, L. F. Keller, K. J. Jeffery, and M. W. Bruford. 2006. Extra-pair fertilization and effective population size in the song sparrow *Melospiza melodia*. *Journal of Avian Biology* **37**:572-578.
- Pollak, J. P., R. C. Lacy, and J. D. Ballou. 2002. *Population management* 2000. Chicago Zoological Society, Chicago.
- Ralls, K., P. H. Harvey, and A. M. Lyles. 1986. Inbreeding in natural populations of birds and mammals. Pages 35-56 in M. E. Soulé, editor. *Conservation biology—the science of scarcity and diversity*. Sinauer Associates, Sunderland, Massachusetts.
- Slate, J., P. David, K. G. Dodds, B. A. Veenvliet, B. C. Glass, T. E. Broad, and J. C. McEwan. 2004. Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. *Heredity* **93**:255-265.
- Soulé, M. E. 1980. Thresholds for survival: maintaining fitness and evolutionary potential. Pages 151-169 in M. E. Soulé and B. A. Wilcox, editors. *Conservation biology—an evolutionary-ecological perspective*. Sinauer, Sunderland, Massachusetts.
- Toro, M., J. Rodriganez, L. Silio, and C. Rodriganez. 2000. Genealogical analysis of a closed herd of black hairless Iberian pigs. *Conservation Biology* **6**:1843-1851.
- van Raden, P. M. 1992. Accounting for inbreeding and crossbreeding in genetic evaluation of large populations. *Journal of Dairy Science* **75**:3136-3144.
- Waples, S. R. 2002. Definition and estimation of effective population size in the conservation of endangered species. Pages 147-168 in S. R. Beissinger and D. R. McCullough, editors. *Population viability analysis*. University of Chicago Press, Chicago.
- Wheelwright, N. T., C. R. Freeman-Gallant, and R. A. Mauck. 2005. Asymmetrical incest avoidance in the choice of social and genetic mates. *Animal Behaviour* **71**:631-639.
- Wright, S. 1922. Coefficients of inbreeding and relationship. *The American Naturalist* **56**:330-338.

