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Genetic structure and mating system in the palila, an endangered Hawaiian honeycreeper, as assessed by DNA fingerprinting

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Abstract

We conducted DNA fingerprinting analyses to ascertain the mating system and population genetic structure of the palila, an endangered Hawaiian honeycreeper, which occupies a fragmented range on the Mauna Kea volcano of the island of Hawai'i. DNA fingerprinting of twelve complete families from the Pu'u La'au population revealed no evidence of extrapair fertilization or intraspecific brood parasitism. Band-sharing coefficients from fingerprints produced with two probes revealed that the large Pu'u La'au population on the southwest slope of Mauna Kea, and a smaller, geographically separate population on the east slope (at Kanakaleonui) had relatively high and virtually identical levels of minisatellite variability (mean S of 0.27 for each population based on combined data of M13 and Jeffreys 33.15 probes). The two populations also had nearly identical allele frequencies based on their mean corrected similarity, S_{ij}, of 0.98. These data suggest that the two populations have not been fragmented long and/or have sufficient current gene flow to ameliorate any affects of genetic drift. We conclude that present levels of inbreeding are low within both populations, and that proposed translocations of individuals from Pu'u La'au to Kanakaleonui appear appropriate from a genetic standpoint.

Keywords: endangered species, DNA fingerprinting, genetic variation, *Loxioides bailleui*, mating system, Hawaiian honeycreeper

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Introduction

Molecular analyses of genetic variation within and among populations can reveal patterns of mating, population structure, and dispersal that may be difficult to determine using the more classical tools of marking and observing individuals. Such genetic data may provide indications of long-term effective population sizes and levels of inbreeding within demes and of gene flow among demes (e.g. Wright 1978; Nei 1987; Rockwell & Barrowclough 1987; Avise *et al.* 1988; Slatkin & Maddison 1989; Neigel *et al.* 1991 for various methods). Endangered species can be useful sources of data to assess theories of population genetic structure: their ranges are often fragmented and their populations reduced. In addition, management of endangered species often requires information about the levels of genetic fragmentation and variation, as well as the amounts and effects of inbreeding within the population (Soulé 1980; Templeton *et al.* 1990; Hedrick & Miller 1992).

Both theoretical and empirical analyses indicate that small and declining populations lose both heterozygosity and allelic diversity (Wright 1931; Nei *et al.* 1975; Maruyama & Fuerst 1985; Lande & Barrowclough 1987). However, the rate of reduction in allelic diversity is often much higher because of the loss of rare alleles which do not contribute greatly to heterozygosity (Maruyama & Fuerst 1985; Allendorf & Leary 1986). Moreover, if populations remain small beyond an initial bottleneck, variation continues to be lost because of continued consanguineous mating (Wright 1931; Nei *et al.* 1975). This loss can have negative consequences for fitness, both from the increased probability of inbreeding depression and because lost variation may not be available for subsequent adaptation (Allendorf & Leary 1986; Lande & Barrowclough 1987).

Most previous studies of genetic structure of populations have used allozyme or mtDNA restriction fragment length polymorphisms as genetic markers. For many of these comparisons, variability is so close to zero that accurate assessments of either variability or structure can be difficult (Powell 1983; Wayne *et al.* 1986). The method of DNA fingerprinting uses repetitive DNA probes that hybridize to hypervariable minisatellite loci to assay presumably genome-wide levels of variation (Jeffreys *et al.* 1985; Burke 1989). Their high mutation rates and variability make them potentially useful markers for examining the effects of recent population history on genetic variation (e.g. Gilbert *et al.* 1990, 1991). Calibrations with various parameters of demography and relatedness justify their use (Kuhnlein *et al.* 1990; Gilbert *et al.* 1991; Reeve *et al.* 1992), and recently developed statistical methods allow direct estimates of relatedness and variables of population genetic interest (Jeffreys *et al.* 1985; Lynch 1988, 1990, 1991; Jin & Chakraborty 1993, 1994).

The palila (*Loxioides bailleui*) is an endangered Hawaiian honeycreeper (Fringillidae: Drepanidinae) presently limited to the island of Hawai'i. Palila are known from fossil excavations to have been present on at least one other of the main Hawaiian Islands (Oahu Island) prior to Western contact (Olson & James 1982; James & Olson 1991). Extensive range contraction on Hawai'i has been documented during the past 100 years (Banko 1986), and the species now survives only on the dormant volcano, Mauna Kea (Fig. 1). Here palila occupy an estimated 13 900 ha of woodland dominated or codominated by the mamane tree (*Sophora chrysophylla*) (Scott *et al.* 1984). Unripe mamane seeds comprise the palila's principal food. Mamane woodlands on Mauna Kea were severely depleted by feral sheep, cattle and goats until the 1980s when most of the animals were removed; the vegetation is now recovering in most areas. The palila population presumably declined along with habitat degradation. Annual population estimates for 1975–92 fluctuated within a range of 1600–6400 palila (Scott *et al.* 1984; and National Biological Survey, Hawai'i Field Station, unpublished data). The majority of the population occupies a large expanse of woodland on the western slope of the mountain, while the remainder of the birds are patchily distributed across the southern and eastern slopes (Fig. 1). Thus, the palila population is both reduced in size and fragmented.

In this paper we report on DNA fingerprinting data collected to assess both the genetic mating system and the genetic structure of the palila metapopulation on Mauna Kea volcano. Specifically, we examine genetic variation within two populations at opposite ends of the palila distribution on Mauna Kea and the level of genetic differentiation between them. We compare the level of genetic variation in the palila to that of the abundant common 'amakihi (*Hemignathus virens*) from the same location. We also document an absence of extrapair fertilization or intraspecific brood parasitism within a sample of families from one part of the palila population. Last, to assess the possibility that birds are pairing with relatives in the population, we compare genetic similarity within mated pairs to genetic similarity within random pairings from the same population.

Methods

Field protocol

Blood samples were collected from palila at two principal sites, Pu'u La'au and Kanakaleonui, on the western and eastern slopes, respectively, of Mauna Kea (Fig. 1). We sampled palila at four banding stations at Pu'u La'au during routine banding from January 1989 to October 1991 and at nests in 1990, 1991 and 1993, where we attempted to sample all family members. We also sampled birds at a temporary banding station at Kanakaleonui (Fig. 1) in February and March 1990. At both sites, many of the incidentally captured common 'amakihi were also sampled (see below).

Adults were captured in mist nets set at 1–5 m above the ground. Nestlings were banded and bled when 13–18 days old. Handling time was kept to < 30 min, and nestlings were returned to nests immediately after sampling. Rather than bleeding some nestlings in 1990, we instead took a developing (blood) feather from the upper wing coverts. Unfortunately we were unable to obtain sufficient DNA for fingerprinting from most of the feathers collected from nestlings, but the degraded nature of many

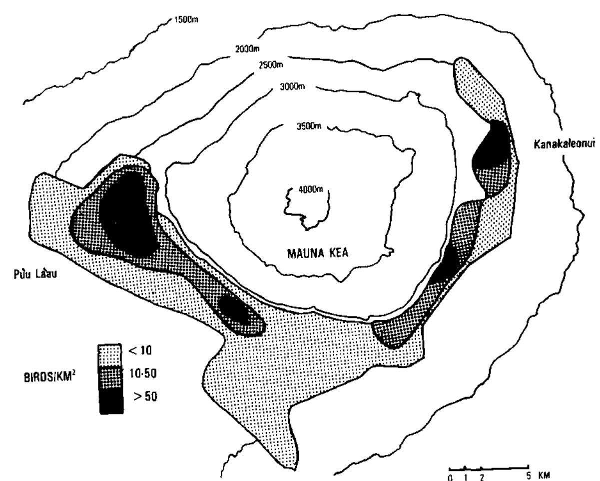

Fig. 1 Map of Mauna Kea Volcano, Hawai'i Island, showing the range and relative abundance of the palila. Shown on the map are the two main sampling areas: Pu'u La'au and Kanakaleonui. The map is modified from Fig. 95 of Scott *et al.* (1986).

of the sample preparations suggested storage problems in the field. We did not collect samples from fledglings or from second-year palila, nor from females that were incubating or caring for nestlings < 13 days old. Birds were bled from the wing vein into microvettes containing EDTA according to the protocol of Wingfield and Farner (1976). Samples ranged from 25 to 200 μL in volume, depending on the age or size of the birds. Blood was mixed with an equal amount of SSC or lysis buffer (tris-EDTA-SDS) and refrigerated during transport to camp, where the samples were placed in liquid nitrogen.

To test the effect that bleeding had on short- and long-term survival of palila, we conducted controlled experiments on this species and on the smaller common 'amakihi. For each bird that was bled, we selected another of the same sex and age class that was captured on the same day (or as close in time as possible) as the bled bird to serve as a control. Birds not bled were otherwise treated the same as birds bled. One-tailed Fisher's exact tests compared numbers of bled and control birds that were subsequently recaptured or not recaptured after 1 month, 6 months and 1 year. We used one-tailed tests to gain power for testing the hypothesis that a smaller percentage of bled birds than control birds would be recaptured in subsequent periods.

Laboratory analyses

Blood samples were brought to the laboratory in liquid nitrogen and stored at –70 °C until DNA isolation. DNA was extracted from blood cells by cell lysis, proteinase K digestion, equilibrated phenol-chloroform extractions, and ethanol precipitation. DNA was spooled onto glass rods and dissolved in 1 × Tris-EDTA. The DNA was then dialysed against TE and stored in microfuge tubes at –20 °C. Each DNA sample was fluorescence-quantified by ethidium bromide staining in a 0.8% agarose mini-gel in 1 × TBE.

Up to 10 μg of each sample was digested overnight with 10 units/μg of restriction endonuclease *Hae*III to ensure complete digestion. The samples were electrophoresed in a 1% agarose mini-gel to check the extent of digestion, as well as to reconfirm DNA concentrations. We attempted to place the same amount of DNA (about 4–5 μg) in each lane. The digested DNA samples were run in a 20-cm × 30-cm 1% agarose, 1 × TBE gel for 40 h at about 50 V. The gel was stained with ethidium bromide and photographed, and the 'supermarker' (Bio-Synthesis Corporation) size standard was marked onto the gel surface with a pipet tip.

DNA in the gel was depurinated in 0.25 M HCl, denatured in 0.5 M NaOH – 1.5 M NaCl, and neutralized in 20 × SSC. The DNA was then transferred to a nylon filter (MSI-NT) by vacuum or pressure blotter for 2–3 h in 20 × SSC transfer buffer. The 'supermarker' was marked on the nylon filter by puncturing through the gel impressions into the filter with a straight pin. DNA was bound to the filter by either baking at 80 °C for 2 h, or UV cross-linking. Protocol for hybridization of M13 and Jeffreys 33.15 probes to DNA on the filter followed that of Westneat *et al.* (1988), with overnight hybridizations at 60 °C for M13 and 65 °C for Jeffreys 33.15. Probes were labelled by the random hexamer method with [α^{32}P]dCTP to an activity greater than 8×10^8 CPM/μg of probe DNA. The holes on the filter indicating the 'supermarker' were painted with light-emitting 'glo-juice' (IBI). Exposures to Kodak XRP film with Cronex Lightning Plus intensifying screens varied from overnight to several days; usually more than one exposure was needed to resolve both large and small fragments.

Data analysis

Families were analysed by placing digested DNA samples of adult nest attendants in the outer lanes and those from nestlings inbetween. Individuals not in families were arbitrarily positioned on gels, some of which were specifically designed to assess variation within particular populations or species. To compare the Pu'u La'au and Kanakaleonui samples directly, samples from birds from each location were run in alternate lanes within a single gel, as recommended by Lynch (1990).

Autoradiographs were scored first to confirm that all fragments within a nestling profile were present within the profiles of its putative parents. Nestlings were excluded from a pair if more than two fragments were present in the offspring but not in either adult. We presumed, as did Westneat (1990), that only one or two extra fragments in a nestling fingerprint profile were the result of an artifact or mutation rather than extrapair fertilization or intraspecific brood parasitism. We defined related birds as those sibling or parent-offspring dyads for which the offspring could not be excluded from putative parents by more than two extra fragments. A mutation/artifact rate was calculated as the number of such extra fragments divided by the sum of all fragments in the offspring profiles.

The number of fragments greater than about 2kb in size within each lane was counted. A presence-absence matrix of same-sized fragments was constructed for each gel. The number of fragments shared between profiles was counted and used to calculate the coefficient of band-sharing or similarity (S of Lynch, 1990): $S = 2N_{xy}/N_x + N_y$. Here, N_{xy} is the number of fragments shared between the profiles of individuals x and y, and N_x and N_y are the number of fragments in the profiles of individuals x and y, respectively. Band-sharing coefficients were calculated for each pairing of individuals within families and also

for pairings of putatively unrelated individuals when possible. We calculated the mean and an uncorrected standard deviation of S for various groupings of individuals (i.e. related and unrelated Pu'u La'au, Kanakaleonui, 'amakihi). We used mean S, mean number of fragments per profile and mutation rate to calculate upwardly and downwardly biased, long-term effective population sizes for each population (Lynch 1991, eqns 5 and 10).

Genetic differentiation between the two study populations was determined by calculating S'_{ij} for all pairwise comparisons of individuals from Pu'u La'au with those from Kanakaleonui. These values were averaged and used to calculate a between-population genetic similarity corrected for within-population similarity (S_{ij}; Lynch 1990, eqn 11) and an estimate of F_{st} (Lynch 1990).

Results

Samples and effect of bleeding

We sampled a total of 124 palila: 45 from banding stations at Pu'u La'au, 10 from the banding station at Kanakaleonui, and 69 from nests at Pu'u La'au, representing 21 complete and incomplete (i.e. missing one parent) families and some associated individuals. We took feathers rather than blood from nestlings of seven of these families, but we did not include these individuals, nor the three families sampled in 1993, in the analysis of survival. We sampled 146 common 'amakihi. For adult or nestling palila, we detected no effect of bleeding on survival (Table 1). Recapture rates of 'amakihi were similar between bled and unbled birds after 1 month and 6 months, but significantly fewer bled 'amakihi than expected were recaptured after one year (Table 1).

Table 1 Effects of blood sampling on recapture rates of two species of Hawaiian honeycreepers

	n	Percentage of birds recaptured		
		> 30 days	> 182 days	> 365 days
Palila adults				
Controls	58	17.2	13.8	8.6
Bled	58	25.9	19.0	12.1
P^a		0.91	0.84	0.82
Palila nestlings				
Controls	145	15.2	10.3	4.8
Bled	19	5.2	0.0	0.0
P		0.17	0.14	0.42
'Amakihi adults				
Controls	146	21.9	15.1	10.3
Bled	146	16.4	11.0	4.1
P		0.15	0.19	0.03

[a]Significance of result from one-tailed Fisher's exact test.

Summary of DNA fingerprint analyses

A total of 70 palila were DNA fingerprinted among seven gels, including 12 complete and three incomplete family groups. Some individuals were run more than once on a gel, or on more than one gel. An eighth gel was devoted to a random sample of 'amakihi from the Pu'u La'au site. An example of an M13-probed fingerprinting gel composed of palila from Pu'u La'au is exhibited in Fig. 2.

Within-population variation was assessed for related (n = 50 comparisons of 42 individuals) and putatively unrelated birds (n = 56 comparisons of 40 individuals) from the Pu'u La'au sample, and putatively unrelated birds (n = 45 comparisons of 10 individuals) from the Kanakaleonui site. One gel (with seven Kanakaleonui and eight Pu'u La'au samples) was designed to assess inter-population variation. Six gels were probed with both M13 and Jeffreys 33.15 minisatellite probes. One other gel, which included a family with two nestlings, was probed only with M13, and these individuals are included only in the family summary below. For the Pu'u La'au sample (n = 60 individuals), a mean (± SD) of 12.2 ± 2.9 and 17.9 ± 5.1 fragments above 2 kb in size were scored following hybridization to M13 and Jeffreys 33.15 probes, respectively. For the Kanakaleonui sample (n = 10 individuals), the mean number of fragments (± standard deviation) was 13.0 ± 1.8 for M13 and 15.0 ± 2.7 for Jeffreys 33.15. We estimated the average number of loci (L of Lynch 1991) for the Pu'u La'au sample as 6.5 for M13 and 9.8 for Jeffreys 33.15. These estimates require the assumptions of no multifragment alleles (i.e. linkage, see below),

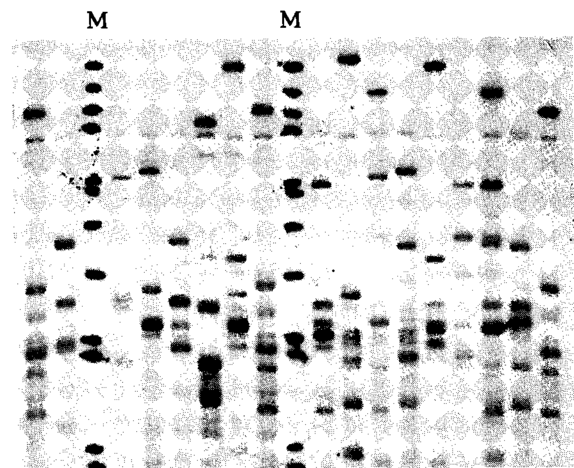

Fig. 2 Example of a DNA fingerprint of 17 palila samples (15 individuals) from the Pu'u La'au study site. DNA was digested with *Hae*III restriction enzyme; the membrane was probed with α^{32}P-labelled M13 DNA. 'M' denotes lanes 3 and 10 containing the 'supermarker' size marker, marked with 'glo-juice' on the filter (see Methods for details). Lanes 1, 9 and 19 contain the same individual.

Table 2 Mean and standard deviations (uncorrected for non-independence) of band-sharing coefficients (S) for 'amakihi and palila. N is the number of dyads. See text for the number of individuals

Location/Species (N)	Mean S	SD
Pu'u La'au		
'Amakihi (66)		
M13	0.173	0.102
J33.15	0.264	0.101
Combined	0.231	0.075
Palila (56)		
M13	0.250	0.115
J33.15	0.304	0.113
Combined	0.270	0.096
Palila-related (50)		
M13	0.584	0.117
J33.15	0.598	0.109
Combined	0.594	0.081
Kanakaleonui		
Palila (45)		
M13	0.232	0.117
J33.15	0.301	0.143
Combined	0.266	0.111

no null alleles (i.e. fragments run off of the gel) and Hardy–Weinberg equilibrium.

Band sharing generally was low for both probes for unrelated individuals in comparison to related individuals (Table 2, Fig. 3). In all comparisons, we found that blots probed with M13 resulted in lower values of S than those probed with Jeffreys 33.15. In one comparison of two putatively unrelated individuals from Pu'u La'au, we found a very high value of S (0.69 for both probes combined), suggesting that the two individuals may be first-order relatives. There was virtually no difference in mean S among different classes of relationship: for female offspring, 0.60 ± 0.07; for male offspring, 0.59 ± 0.09; and for siblings, 0.59 ± 0.08. We detected no obvious associations of fragments across generations within profiles of family groups. This, along with observations that the range and standard deviation of S between siblings are not larger than those of the other related and the unrelated classes (Table 2), suggests that most fragments are not linked. However, additional comparisons within and across generations are needed to completely exclude the existence of multifragment alleles (Brock & White 1991).

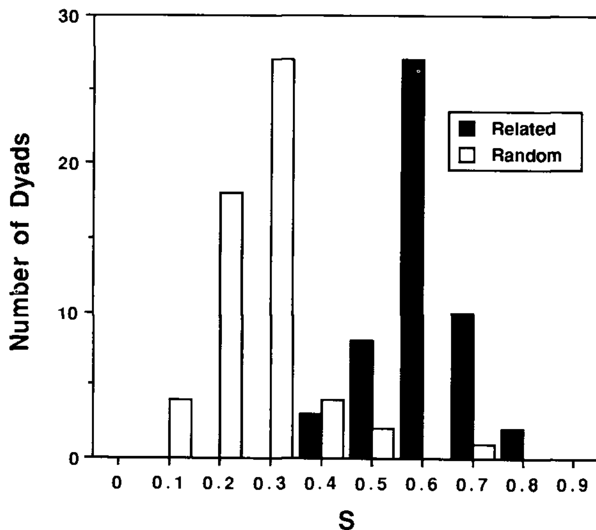

Fig. 3 Distribution of band sharing coefficients (S) for palila from the Pu'u La'au population for both the M13 and Jeffreys 33.15 probes combined. Clear bars represent putatively unrelated (i.e. randomly sampled) pairs of birds; dark bars represent pairings of related individuals (40 parent offspring and 10 sibling–sibling pairs). All related individuals were confirmed by a lack of excluding extra fragments (see text).

Mating system

DNA fingerprinting revealed no positive exclusions of 20 nestlings from their putative parents within 12 complete family groups assessed. For one of these family groups, with two nestlings, we only had data for the M13 probe. A total of eight of the 20 offspring profiles did contain extra fragments (one from M13 and seven from Jeffreys 33.15 fingerprints). Values of S with putative parents were greater than 0.50 for all but one of the seven individuals with exclusionary fragments (and that individual had an S of 0.41 with its mother and 0.70 with its father). The calculated mutation/artifact rate overall was 0.014 per gamete per generation, but was about five times higher for Jeffreys 33.15 (0.021) in comparison to that of M13 (0.004).

We follow the reasoning of Westneat (1990) and others who believe that these uncommon extra fragments likely result from artifacts or novel mutations. In the case of the results for the Jeffreys 33.15 probe, most of the extra fragments may be artifacts: the majority of them were faint bands and may have been missed in the parental profiles, especially if the offspring were homozygous and the parents were heterozygous for the fragment. There was no evidence of incomplete digests or band shifts (based on internal size standards). The M13-generated fingerprints had very few faint fragments (Fig. 2).

To further our view that the extra fragments are not evidence of non-parentage, we assayed the number of excluding fragments found when offspring profiles were compared to profiles of 'random' individuals (i.e. profiles in the lane immediately following the family group on the gel) rather than the attending male. For the sample of attending males the mean number of excluding fragments was 0.4 ± 0.5 (range 0–1). For the sample of 'random' profiles the mean number of excluding fragments was

7.9 ± 2.1 (range 5–12). These data offer additional empirical support to the view that no extrapair fertilizations occurred within the nests sampled.

Genetic structure

Within-population genetic variability was assessed by calculating the average band-sharing among individuals within a given population; populations with a great deal of variability should have low 'background' values of S. Mean values of S, for both M13 and Jeffreys 33.15 probes and combined, were nearly identical between the random samples from Pu'u La'au and Kanakaleonui (Table 2). These values are within the range found typically for outbred taxa using the same probes with similar laboratory protocols (e.g. S for 'amakihi in Table 2; Burke and Bruford 1987, Burke *et al.* 1989, Meng *et al.* 1990, Westneat 1990, Brock and White 1992, Oring *et al.* 1992, Stutchbury *et al.* in press, etc.). 'Amakihi do appear to have slightly higher genetic variability than palila (Table 2).

Within-population variability for the sample of related palila (i.e. comparisons of siblings or parents and offspring) revealed a considerably higher level of band-sharing for both probes (Table 2), but one which is in line with that found for first-order relatives in other taxa (e.g. Burke *et al.* 1989, Westneat 1990, Gibbs *et al.* 1990, Brock and White 1992). Thus there is no indication that palila have less variability at these minisatellite loci than non-endangered species of birds. In addition, we found that individuals within mated pairs appear not to be more closely related than those sampled from the population at large: the level of band-sharing between individuals of mated pairs of palila is similar to that of the overall Pu'u La'au population (average S of 0.273 ± 0.050 for 13 pairs versus 0.270 ± 0.096 for the unrelated sample from Pu'u La'au; Table 2).

Pu'u La'au and Kanakaleonui exhibited almost identical levels of band-sharing for each probe and both probes combined (Table 2), indicating that they do not differ in their levels of genetic variation. In addition, we used the single composite gel of Pu'u La'au and Kanakaleonui samples to estimate an unbiased heterozygosity for each probe and location (Jin and Chakraborty 1993). These estimates require the assumptions that each fragment represents an allele in either a homozygous or heterozygous state and that there is not linkage among fragments. Calculations are based on the frequencies of particular fragments, which are best assayed on a single gel. For M13, we estimated an H (heterozygosity) of 91.1% for Pu'u La'au and 88.7% for Kanakaleonui. For Jeffreys 33.15 we estimated an H of 81.2% for Pu'u La'au and 79.8% for Kanakaleonui.

We calculated long-term effective population sizes using eqns 5 and 10 from Lynch (1991). Both eqns require estimates of mean S and mutation rates as calculated above for each probe (assumed here to be mutation alone). Equation 5 also uses the mean number of fragments. Equation 10 provides a downwardly biased estimate, and eqn 5 an upwardly biased one (Lynch 1991). Lynch suggests that the two together provide an approximation of the range of effective population size, with the assumption that the populations are in drift-mutation equilibrium. Because of the high mutation rate estimated for Jeffreys 33.15 fingerprints (also higher than the typical range of 0.001 to 0.01), the predicted N_e for Pu'u La'au was much lower (27 to 61) than estimates based on M13 fingerprints (188 to 406). We feel that our calculated mutation rate for M13 fingerprints may be less artifactual, and thus suggest that an N_e of 200 to 400 individuals seems a reasonable estimate.

The amount of between-population genetic differentiation was estimated by calculating the corrected mean of S_{ij} (Lynch 1990). This value for the comparison between Kanakaleonui and Pu'u La'au is 0.98 overall (0.99 for Jeffreys 33.15 and 0.97 for M13), indicating that the two populations do not differ greatly in allele frequencies. The calculation of F_{st} (Lynch 1991, eqn 17) over both probes combined is 0.026 (0.004 for Jeffreys 33.15 and 0.040 for M13). We calculated the rate of migration (m) from the island model equation $F_{st} = 1/[4N_e(m + \mu) + 1]$ (Hartl & Clark 1989). We used as parameter values the ranges for N_e of 188 and 406 above, the mutation rate of 0.004, and the F_{st} of 0.040, all based on the results for M13. The range of m for these effective sizes was 1.1–2.8% migrants exchanged per generation.

Discussion

Effect of bleeding

Although bleeding appeared to have no effect on survival of adult or nestling palila, the proportion of bled adult amakihi was lower for all time periods, significantly so after one year (Table 1). Although cause for concern, we believe this latter result may be an artifact of conducting multiple statistical tests and not controlling the overall Type 1 error rate. We cannot otherwise explain these results, for a bird that survives the first six months after being bled would be expected to have the same probability of surviving 12 months as a bird that was not bled. No previous studies of birds that we could locate revealed an effect of blood sampling on survivorship (summarized in Oring *et al.* 1988).

Mating system

Our results, although limited by small sample sizes of nests and nestlings, suggest that neither extrapair fertilization or intraspecific brood parasitism occur commonly

in this population of the palila. We found that none of 20 nestlings could be excluded from their putative parents. The actual frequency of such an event in the population could be higher than the observed 0% given a larger sample of nests or nestlings, but is not likely to be higher than 10–15%. However, we did not want to risk sampling a large number of nests until the long-term impact of such sampling was known.

The negative findings do not conform with the generally high rates of extrapair fertilization that have been recently documented for most putatively monogamous passerine birds (e.g. Westneat 1987, 1990; Wetton *et al.* 1987; Sherman & Morton 1988; Petter *et al.* 1990; Morton *et al.* 1990; Stutchbury *et al.* in press; but see Gyllensten *et al.* 1990, Birkhead *et al.* 1990). These parentage studies, however, have been carried out with species in temperate regions, and tropical, nonmigratory species may differ. To our knowledge, the only other analyses of genetic mating system in socially monogamous birds in the tropics also found no evidence of extrapair fertilization or intraspecific brood parasitism (in common mynas, *Acridotheres tristis*, in Hawai'i, Telecky 1989; and dusky antbirds, *Cercomacra tyrannina*, in Panama – Morton, Tarr, Sangmeister and Fleischer, in preparation).

A number of factors may help to understand our finding of no intraspecific brood parasitism or extrapair fertilization in palila. First, mating opportunities may be severely limited for male palila and threat from extrapair fertilization may be high: the adult sex ratio is heavily biased towards males (National Biological Survey, Hawai'i Field Station, unpublished data), and males show delayed plumage maturation and delayed breeding (for 1989–91 there were no males breeding at 2 years of age (of 105) versus 12.5% of 112 females; $G = 19.0$, $P < 0.001$). Secondly, males defend the nest and female only, not a large territory, and mate guarding has been observed in the vicinity of the nest (van Riper 1980; T. Pratt, personal observation). Thus males may be able to more adequately guard their females against other males than if they defended larger territories. Alternatively, females may have little impetus to obtain copulations from floater or neighboring males since they already are likely to be mated to a an older, high quality male (Smith 1988). The fact that most pairs with surviving individuals renest together, both within and between years (T. K. Pratt, unpublished data), would seem to support this view. Last, the small clutch size of palila (mode of two eggs; van Riper 1980 and this study), and therefore short laying period, may facilitate mate guarding by males and help to thwart extrapair fertilization attempts by neighboring or floater males.

Likewise, females probably do not have much opportunity for intraspecific brood parasitism. Females committing intraspecific brood parasitism would seem to be less likely to synchronize their laying to fit the 1–2-day opening during which they can lay eggs in a neighboring nest and expect them to hatch. In addition, because of the rigorous, cold climate in their high altitudinal range, palila females do not leave nests open to potentially parasitizing females for much time, and both males and females aggressively respond if a foreign female approaches the nest (T. K. Pratt, personal observation).

Genetic variation and inbreeding

Low hatchability of palila eggs (van Riper 1980; Pletschet & Kelly 1990; National Biological Survey, unpublished) has been postulated to be a result of inbreeding depression. Our results indicate that inbreeding and its associated depression is unlikely because of the low relatedness of individuals in mated pairs and the relatively high intrapopulation genetic diversity and effective population size estimates. The former finding is potentially biased because we sampled only successful nests; pairs with nests in which eggs did not hatch could have been more closely related than the successful pairs. Such a pattern was found for Puerto Rican parrots (*Amazona vittata*) by Brock & White (1992).

However, the high genetic variability we found suggests that recent bottlenecks and current population structure have not greatly increased the level of inbreeding, and that inbreeding depression is not likely to be occuring in this population. We should point out, however, that because of the high mutation rates of minisatellite loci in comparison to more classical loci, we cannot rule out that significant variation has not been lost from other loci. Our preliminary comparisons indicate that control region sequences of mitochondrial DNA for palila may be less variable than for other honeycreeper taxa, suggesting perhaps, some effect of a bottleneck (although mtDNA is considered more susceptible to bottleneck effects than nuclear).

One interesting finding was that mutation/artifact rates differed between the two probes. We stated above that we felt this may be caused by more artifacts in the Jeffreys 33.15 fingerprints. But assuming the difference is due to mutation, why would the rate not match the direction of variation? If most of the mutations occur only at one or two loci, while the rate is much lower for others, accumulation or retention of variation may be lowered (i.e. very high heterozygosity at only one or two loci might not ameliorate the effects of lower heterozygosity at other, less mutable, loci).

Because of the estimated small size and putative degree of isolation of the Kanakaleonui population, we would predict that the Kanakaleonui population should have lower genetic variability (through inbreeding) and should have different allele frequencies (through genetic

drift) when compared to the larger Pu'u La'au population. In addition, breeding dispersal distances in the Pu'u La'au population appear to be very small based on radiotracking data and recaptures of banded individuals (Fancy *et al.* in press), although few data on natal dispersal are available. Our results indicate that the palila populations at Pu'u La'au and Kanakaleonui do not differ in either their levels of variability (as measured by band-sharing) nor in their allele frequencies (as measured by the corrected similarity of 0.98 and the F_{st} of 0.026).

We suggest two possibilities to explain this discrepancy. First, the palila populations on Mauna Kea may have been connected until only recently, thus not allowing time for the effects of inbreeding and drift to occur. This seems unlikely given our results for the introduced populations of the related Laysan finch (*Telespiza cantans*) on islets in the Pearl and Hermes atoll (Fleischer *et al.* 1991; Tarr, Fleischer and Conant, unpublished). In this metapopulation, four isolated populations within the size range of the one at Kanakaleonui show much lower variability and have apparently drifted greatly in allele frequencies over only the past 25 years, especially in comparison to their large source population on Laysan Island. Alternatively, natal dispersal of palila may be high, thus ameliorating any effects of local inbreeding or drift between the two populations, or the Kanakaleonui population may be a population only recently founded by birds from the western Mauna Kea population following a local extinction there.

Conservation and management implications

Our results indicate that there should be little concern at the present time that palila populations on Mauna Kea are inbreeding to any significant degree. In addition, they suggest that the population decline that has apparently occurred during the past century has not appreciably reduced the minisatellite variability in the palila populations, even relative to that of a more abundant and related Hawaiian honeycreeper, the common 'amakihi. However, comparisons of mitochondrial DNA, single-copy nuclear and microsatellite DNA variation between current and older museum specimen populations of palila should provide additional important data to assess this question. If palila populations decline more, or develop a genetic structure of very small, isolated populations, we may find it useful to re-evaluate their levels of genetic variability. The lack of differentiation in minisatellite frequencies between the Pu'u La'au and Kanakaleonui populations also implies that planned translocations to the smaller population would not likely result in significant genetic change and would likely reduce the vulnerability of the smaller population to extinction by demographic factors.

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This paper is the first result from a collaboration between researchers from the Molecular Genetics Laboratory at the National Zoological Park and the Hawai'i Research Group of the National Biological Survey. Rob Fleischer is Head of the Molecular Genetics Laboratory and an evolutionary geneticist. He has been studying the genetics of various Hawaiian birds for almost ten years. Cheryl Tarr is a research assistant in the Molecular Genetics Laboratory, and a graduate student in molecular evolution at Pennsylvania State University. Her dissertation research involves the systematics and population genetics of Hawaiian honeycreepers and other endangered Hawaiian taxa. Thane Pratt is a research biologist with the Hawai'i Research Group of the National Biological Survey. His primary interests are in the ecology, behavior and conservation of native birds of Hawai'i and elsewhere in the Pacific. He, along with other researchers of the Hawai'i Field Station, has just completed a long-term study of population ecology of the palila.