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Genetic and morphological differentiation between remnant populations of an endangered species: the case of the Seychelles White-eye

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The Seychelles White-eye *Zosterops modestus* is a critically endangered species that survives in two remnant populations on the islands of Mahé and Conception. Multilocus minisatellite DNA fingerprinting and morphometric measurements were used to assess the levels of variation between these populations. Mahé White-eyes are on average significantly larger than Conception birds, as are males compared to females. The mean level of bandsharing (*c.* 60%) indicates low levels of genetic variability within both populations. Bandsharing is significantly lower between populations (32%), suggesting that the two populations are genetically isolated from each other, and that one is not a subsample from the other. Both populations should therefore be considered as equally important genetic reservoirs that deserve to be safeguarded. Although each population appears locally adapted to its particular island environment, such limited differentiation is not considered of major taxonomic significance. Recombining genetic variation by mixing individuals from both populations may be beneficial for the species in view of future island transfers. This illustrates the importance of investigating differentiation between remnant populations of a threatened species to orientate future conservation and management measures.

The Seychelles White-eye *Zosterops modestus* is a critically endangered species endemic to the Seychelles archipelago (Collar *et al.* 1994, BirdLife International 2000) where it was, until recently, known only from the main island of Mahé. During the 19th century (Newton 1867) and the early 20th century (Nicoll 1906) the White-eye was locally abundant on Mahé. It then underwent a drastic decline and was thought to be extinct for several decades (Crook 1961), until it was rediscovered (Loustau-Lalanne 1962). In the 1970s White-eyes were estimated to be fewer than 100 in number and declining (Watson in Collar & Stuart 1985). Between 1995 and 1997, intensive searches coordinated by the ministry of Environment and BirdLife International showed that the number and distribution of White-eyes had declined by approximately 50% since 1975–77 (Rocamora 1997b). The Mahé population was estimated at *c.* 50 individuals.

In 1996, following repeated public appeals through the media, a population was reported on Conception (4°39′S, 55°22′E), a small island (63 ha) 1.6 km off Mahé (4°38′S, 55°25′E). The existence of this population was confirmed (L. Chong-Seng pers. obs.) and the island was estimated to host 30–50 breeding pairs and at least 200 birds (Rocamora 1997a, 1997b). This discovery brought renewed hope for the future of this species and a two-year research project was started in 1998 as the first phase of the Seychelles White-eye Recovery Programme. This study provided basic information on the ecology and breeding biology of the Conception population (Rocamora & François 2000). Some information is also available from the small population on Mahé (e.g. Loustau-Lalanne 1962, Feare 1975, Mellanby *et al.* 1996, Rocamora 1997a, 1997b), where the very high rate of nest failure – mainly due to introduced rats and birds – has been identified as the main cause of its decline (Rocamora & François 2000).

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It is important to determine whether differences exist between the Conception and Mahé White-eyes, as this will have implications for the future management and conservation of this species. The Seychelles White-eyes' history of isolation, the severe population bottleneck that occurred during the last century and the limited size of both populations suggest that this species is highly inbred and may contain limited genetic variation. It is important to quantify genetic variation as reduced variation can lead to inbreeding depression and reduced population viability, and is believed to increase the probability of extinction in small populations (e.g. van Noordwijk 1994; Frankham 1998, Saccheri *et al.* 1998). Levels of genetic variation shared by the two populations will also help us understand their history and relationship, i.e. is the Mahé population a subset of the Conception population maintained by interisland immigration? Does the Conception population originate from a few Mahé birds that colonized the island in recent times?

In this paper, we investigate morphological differences (including sexual dimorphism) and levels of genetic variation within and between the two remaining populations of Seychelles White-eyes.

METHODS

Birds were caught in mist-nets placed next to fruiting trees (Conception) or lured into the nets using a tape playing previously recorded White-eye vocalizations (both islands). Body measurements (wing length, maximum tarsus length (Redfern & Clark 2001), bill, head plus bill, tail and mass) and plumage coloration were recorded. We were unable to sex and age birds in the hand from plumage or morphometric differences.

Birds on Conception (192) were ringed with Paris Museum rings whereas those on Mahé (35 since 1996) were ringed with British Trust for Ornithology rings. All birds were also colour-ringed. Colour-ring combinations used on Mahé and Conception were distinctive so that a bird from Conception recaptured or resighted on Mahé could always be identified easily.

Approximately 25 µL of blood was taken from captured birds by piercing the brachial vein with a small hypodermic needle and then drawing off the blood using a graduated capillary tube. This sampling technique has been shown to have no ill effects on birds (Stangel 1986). The blood was immediately mixed with at least five volumes of 100% ethanol.

Mean, standard deviation and confidence intervals were calculated for all measurements. To avoid observer error, measurements were taken by a single observer (G.J.R.). Statistical analyses were done using S-plus, Statistica or Excel.

Morphometric comparison

To compare the morphometric measurements between the two islands and between sexes, three different tests and a multivariate analysis were used. The Welch modified two-sample *t*-test compares the means of two normally distributed variables when the variances are unequal (Mood *et al.* 1974, Snedecor & Cochran 1980). Given that we carried out repeated *t*-tests for the two groups of birds (one for each of the seven variables measured), we applied Bonferroni correction (Keppel 1991) to the standard significance level to increase confidence in the significance of the results. To guard against possible deviations from normality, a Monte-Carlo test (Efron & Tibshirani 1993, Davison & Hinkley 1997) was applied. A third way of comparing the samples is to look at the expected difference between individuals chosen at random in each sample. This specifically addresses the probability that a bird from Mahé is larger than a bird from Conception. A Monte-Carlo procedure was devised by random sampling with replacement pairs of birds (one from each of the original samples) and taking the difference between them. The resulting distribution of differences was used to estimate the average difference between individual birds and associated probability statements.

Principal Component Analysis (Cooley & Lohnes 1971, Venables & Ripley 1994) summarizes the relationships between the different measurements. We used the first two components of a PCA to determine the variables that best explain the observed differences between individuals, and to provide a graphical representation of population separation. The extraction of the principal components was based on a covariance matrix, without rotation of the components.

Genetic analysis

Multilocus minisatellite DNA fingerprinting (Jeffreys *et al.* 1985) was used to assess the levels of genetic variation within and between the populations. This technique was used in preference to microsatellite markers as it provides a quicker and more cost

efficient way of investigating levels of genetic variation, especially in species for which microsatellites have not already been isolated or characterized. It has also been applied successfully to studies of other small isolated island populations (Gilbert *et al.* 1990, Degnan 1993, Kappa 1998) with which the Seychelles White-eye can be directly compared.

DNA extractions were conducted as follows. A small (2 mm × 2 mm) piece of hardened blood was removed from the ethanol using a sterilized wooden toothpick, air dried for 10 min and then added to proteinasing solution (1 M TRIS-HCl, pH 8.0; 0.1 M NaCl; 1 mM EDTA) containing 0.5% SDS and 5 units of proteinase K (Sigma). The DNA required for fingerprinting was then extracted using standard phenol extraction methods (Sambrook *et al.* 1989, Bruford *et al.* 1998). The smaller samples of DNA required for sexing were extracted using a quicker ammonium acetate extraction method as follows. The blood/proteinasing solution was digested (with constant agitation) at 55 °C for 3 h; 250 µL of 4 M ammonium acetate was added, then the solution was vortexed and left at room temperature for 15 min. The sample was centrifuged at 14 000 rev/min for 10 min and the supernatant decanted into a clean, labelled eppendorf tube. To precipitate the DNA from the supernatant, 2 volumes of 100% EtOH were added and the sample was centrifuged at 14 000 rev/min for a further 10 min. The supernatant was decanted and the pellet was rinsed in 1 mL of 70% EtOH and air-dried for 30 min. The pellets (from both extraction methods) were dissolved in 300 µL of $1 \times T_{10}E_{0.1}$ (10 mM Tris, 0.1 mM EDTA) by leaving them at room temperature overnight.

Molecular sexing was undertaken using the PCR method (primers P2 and P8) devised by Griffiths *et al.* (1998). PCR products were run on a 3% agarose gel (containing ethidium bromide) for 60 min at 100 V and visualized under UV at 254 nm. Females are the heterogamatic sex in birds and products from both the CHD-w and the CHD N-w genes are amplified in this PCR reaction; consequently, two products of different sizes are visualized on the agarose gel. Males are homogamatic and only produce the band corresponding to the CHD N-w gene.

The Seychelles White-eye has a complex cooperative breeding system (Rocamora & François 2001). Therefore, a subset of birds for DNA fingerprinting was chosen from each island according to strict criteria (only the main adult male and female from a group) to minimize the chance of directly related birds being used in the analyses. Twenty-two putatively unrelated birds were sampled on Conception and 18 on Mahé. Samples were analysed using standard multilocus minisatellite DNA fingerprinting methods following Bruford *et al.* (1998). DNA was digested with *Hae*III and probed with radioactively labelled 33.15 (Jeffreys *et al.* 1985).

Fingerprints were scored (by D. Richardson) following Bruford *et al.* (1998). Two gels, each containing 20 individuals, were run. Individuals from the same island were run in adjacent pairs and pairs from the two islands were alternated. In this way it was possible directly to compare the bandsharing of adjacent dyads of birds (i) from within Mahé, (ii) from within Conception and (iii) one individual from each of the populations. As each individual was used only once in within- and between-population comparisons, all comparisons are independent. In each case, calculation of the bandsharing coefficient was based on Bruford *et al.* (1998). The mean within-population bandsharing coefficient (similarity) was calculated by averaging the bandsharing coefficients of all pairs of individuals sampled in a population. Similarity of bandsharing between populations, corrected for within-population similarity, was calculated using Lynch's (1991) formula:

$$S_{ij} = 1 + S'_{ij} - ((S_i + S_j)/2)$$

where S_i is the mean proportion of bands shared among individuals of population i and S'_{ij} is the mean proportion of bands shared between random pairs of individuals across populations i and j.

RESULTS

Morphometry

Differences between populations

Birds on Mahé have, on average, significantly longer wings, tails and tarsi than birds on Conception (Table 1). For these three measurements, there is a high probability of birds from Mahé being larger than from Conception (varying between 70% and 85%). Mass and bill length are also slightly greater on average on Mahé (0.40 g and 0.30 mm, respectively), the differences being statistically significant ($P < 0.03$) only with the Monte-Carlo test. Only 'bill plus head' length showed no statistically significant difference. After applying Bonferroni correction to the seven *t*-tests conducted (using $P = 0.05/7$), differences in wing length, tail and tarsus measurements were still statistically significant, while differences in

Table 1. Comparison of Seychelles White-eye measurements between Mahé and Conception.

Variable	Sample statistics[a]		*t*-test[b]	Difference between means[c]	Difference between individuals[d]
	Mahé	Conception			
Mass	12.41	12.01	*t*: 1.92	0.39	0.41
(g)	(1.04)	(0.78)	*df*: 49.04	(0.02–0.82)	P(M > C): 0.53
	N = 32	*N* = 65	*P* = 0.06	*P* = 0.018	
Wing length	61.96	59.84	*t*: 6.93	2.12	2.08
(mm)	(1.51)	(1.44)	*df*: 64.16	(1.54–2.69)	P(M > C): 0.77
	N = 35	*N* = 74	*P* < 0.001	*P* < 0.001	
Tail	40.63	39.18	*t*: 5.35	1.45	1.45
(mm)	(1.36)	(1.04)	*df*: 47.72	(0.93–1.97)	P(M > C): 0.70
	N = 33	*N* = 75	*P* < 0.001	*P* < 0.001	
Tarsus 1	17.75	16.88	*t*: 4.90	0.86	0.85
(mm)	(0.94)	(0.57)	*df*: 42.72	(0.54–1.21)	P(M > C): 0.78
	N = 35	*N* = 34	*P* < 0.001	*P* < 0.001	
Tarsus 2	19.94	18.97	*t*: 5.24	0.98	0.97
(mm)	(0.88)	(0.66)	*df*: 62.85	(0.63–1.33)	P(M > C): 0.80
	N = 33	*N* = 75	*P* < 0.001	*P* < 0.001	
Bill	15.69	15.39	*t*: 1.95	0.29	0.29
(mm)	(0.77)	(0.63)	*df*: 51.98	(0.005–0.57)	P(M > C): 0.67
	N = 34	*N* = 74	*P* = 0.057	*P* = 0.025	
Bill & head	29.05	28.88	*t*:1.47	0.17	0.17
(mm)	(0.61)	(0.47)	*df*: 51.87	(–0.06–0.40)	P(M > C): 0.56
	N = 32	*N* = 72	*P* = 0.148	*P* = 0.087	

[a]Values in parentheses indicate standard deviation σ. *N* = number of individuals sampled.
[b]*t*: value of the *t*-statistic; *df*: degrees of freedom; *P*: level of significance of the test.
[c]Values are the mean of the differences from the bootstrap procedure; values in parentheses represent the lower and upper limits of a 95% confidence interval for the difference between means.
[d]Average difference between individuals; P(M > C): Probability that an individual from Mahé is bigger than an individual of Conception island.

Table 2. Factor loadings relative to each measurement variable for the three first components of a PCA, showing how each axis is related to the morphological traits.

	Component 1	Component 2	Component 3
Wing length	0.812		0.498
Tarsus articulation (Tarsus 1)	0.230	0.505	–0.291
Tarsus plus tibia (Tarsus 2)	0.257	0.510	–0.567
Mass	0.211		–0.178
Tail	0.394	–0.647	–0.253
Bill	0.144	–0.220	–0.244
Bill plus head		0.125	–0.340

bill, bill plus head, and mass were not. However, for all the variables compared, there is an overlap between individual measurements as shown by the standard deviations (Table 1). In general the results of the three tests are consistent, with the exception of some differences in *P*-values reported by the *t*-test and the more robust bootstrap procedure for those variables that depart most from the normal distribution. Repeatability values could not be calculated for the different morphological measurements, as White-eyes were only measured once during their capture, and few individuals were recaptured.

The first two components (or axes) of the Principal Component Analysis together explain 73.3% of the total variability (59.3% and 14.0%, respectively). Table 2 shows the factor loadings relative to each variable, i.e. how each of the first two axes is related to the morphological traits. The vectors on Figure 1 are the variables plotted according to their relative contribution on each of the two first components.

Table 3. Comparison between male and female Seychelles White-eye measurements (from both Mahé and Conception islands).

Variable	Sample statistics[a]		*t*-test[b]	Difference between means[c]	Difference between individuals[d]
	Male	Female			
Mass (g)	12.12 (0.72) (*N* = 37)	11.88 (0.70) (*N* = 20)	*t*: 1.251 *df*: 39.84 *P* = 0.22	0.25 (–0.12–0.62) *P* = 0.10	0.27 P(M > F): 0.52
Wing length (mm)	60.85 (1.45) (*N* = 41)	59.82 (1.62) (*N* = 22)	*t*: 2.502 *df*: 39.19 *P* = 0.016	1.03 (0.24–1.82) *P* = 0.008	1.13 P(M > F): 0.63
Tail (mm)	39.76 (1.55) (*N* = 39)	39.40 (0.96) (*N* = 22)	*t*: 1.121 *df*: 58.38 *P* = 0.267	0.36 (–0.27–0.95) *P* = 0.13	0.35 P(M > F): 0.45
Tarsus 1 (mm)	16.96 (0.69) (*N* = 41)	17.23 (0.42) (*N* = 22)	*t*: –1.958 *df*: 60.04 *P* = 0.055	–0.28 (–0.55–0.01) *P* = 0.024	–0.30 P(M > F): 0.28
Bill (mm)	15.57 (0.64) (*N* = 40)	15.54 (0.68) (*N* = 22)	*t*: 0.136 *df*: 40.94 *P* = 0.893	0.03 (–0.32–0.37) *P* = 0.436	–0.005 P(M > F): 0.47
Bill & head (mm)	28.92 (0.45) (*N* = 41)	28.90 (0.56) (*N* = 22)	*t*: 0.156 *df*: 36.06 *P* = 0.877	0.02 (–0.24–0.29) *P* = 0.432	0.017 P(M > F): 0.51

[a]Values in parentheses indicate standard deviation σ. *N* = number of individuals sampled.
[b]*t*: value of the *t*-statistic; *df*: degrees of freedom; *P*: level of significance of the test.
[c]Values are the mean of the differences from the bootstrap procedure; values in parentheses represent the lower and upper limits of a 95% confidence interval for the difference between means.
[d]Average difference between individuals; P(M > F): Probability that a male individual is bigger than a female individual.

Despite the overlap, individuals from both islands appear well separated in this lower-dimension projection, in particular along the first component, which is most heavily influenced by wing length. This axis shows that wing length, tail length and tarsus length best exemplify the differentiation observed between individuals from the two islands. The second axis opposes mainly birds (from both islands) with longer tails to birds with longer tarsi but, with only 14% of the total variance, its importance is minor. Mahé and Conception populations appear clearly differentiated with respect to each of the two major principal components ($t = -9.31$, $df = 52$, $P < 0.001$ and $t = -4.41$, $df = 46$, $P < 0.001$, respectively; values not departing significantly from normality). The third component (11.8% of the total variability) and all the others are of less importance (eigenvalues < 1; Kaiser criterion).

Sexual dimorphism

Male Seychelles White-eyes usually have longer wings than females (63% probability of *c.* 1.0 mm longer, $P < 0.02$ with *t*-test, $P < 0.01$ with Monte Carlo test). However, Bonferroni correction for the *t*-tests suggests that this difference might not be statistically significant. Tarsus length tends to be slightly longer for males on average (0.27 mm, $P < 0.05$ with Monte Carlo test). No other morphological measurements indicated a significant difference between sexes (Table 3). This difference was not observed on Mahé, although the number of females captured (seven) was very low.

DNA analysis

Sexing

Out of 106 birds captured, 74 (70%) were males and 32 (30%) were females. On Mahé, 72% (18/25) were males, while 69% (56/81) were male on Conception; the difference was not statistically significant.

Population genetics

The numbers of 'scorable' bands and the bandsharing coefficients within and between populations are summarized in Table 4. A mean of 17.9 ± 0.54 se fragments in the size range of 3–20 kb was scored per individual. Significantly fewer scorable bands were present in individuals from the Mahé population

Table 4. Average bandsharing coefficients within and between populations of the Seychelles White-eye detected by multilocus minisatellite probe 33.15.

	Scorable bands			Bandsharing coefficients		
	N	Mean	se	Comparisons	Mean	se
Within populations						
Mahé	18	16.45	0.76	9	0.61	0.03
Conception	22	19.72	0.51	10	0.59	0.03
Combined	40	17.93	0.54	19	0.60	0.02
Between populations						
Mahé × Conception	–	–	–	18	0.32	0.02

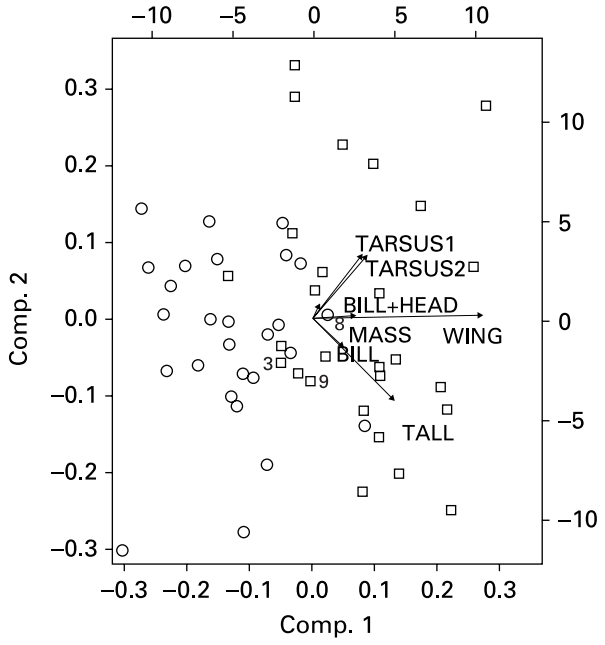

Figure 1. Factorial plan of a Principal Component Analysis showing the separation between Mahé (○) and Conception (□) birds from their body measurements. The vectors are the variables plotted according to their relative contribution on each of the two components.

compared with those from Conception (16.45 ± 0.76 se vs. 19.72 ± 0.51 se, $t = 3.4$, $df = 38$, $P < 0.005$). The mean level of bandsharing was high within both Mahé (0.61 ± 0.03 se) and Conception (0.59 ± 0.03 se) indicating that there is low genetic variability within each population. There was no difference between the populations in the level of within-population bandsharing ($P = 0.61$). However, within-population bandsharing was significantly higher than between-population bandsharing (see Fig. 2, 0.60 vs. 0.32, respectively, *t*-test = 3.4, $df = 38$, $P < 0.001$). Between-population bandsharing, after taking into consideration the within-population bandsharing, was 0.72.

DISCUSSION

Morphological differences

Significant morphological differences exist between the two populations of White-eyes. The Mahé White-eyes have, on average, larger bodies and about an 80% probability of having longer wings, tarsus and tail than Conception White-eyes. Although the difference between the means of these variables is statistically significant, the actual differences are relatively small (wing 3.4%, tail 3.6%, tarsus 4.9%). The overlap between the measurements from the islands makes it impossible to identify accurately the origin of an individual just from its measurements.

Differences in size between populations of the same species across an archipelago are known from other species, and probably occur frequently (e.g. Galapagos finches *Geospiza* sp. Lack 1968, Grant & Grant 1989; Australian Silvereyes, *Zosterops lateralis* Degnan 1993). Smaller body-sizes on Conception could be due to founder effects. Alternatively, the size differences could indicate local adaptation towards the environment on Conception or be due to phenotypic plasticity and reflect, for example, differences in food availability. The smaller size of the Conception White-eyes is also consistent with the general theories of island biogeography that predict smaller bodies in island than in mainland populations, as this allows higher population sizes in limited island habitats and hence increased survival (MacArthur & Wilson 1967, Blondel 1986).

No significant plumage differences could be detected between Mahé and Conception White-eyes. However, we noted that birds from Conception tended to have more uniformly grey upper-parts compared with ones from Mahé, which sometimes show greenish or reddish tones on their back and

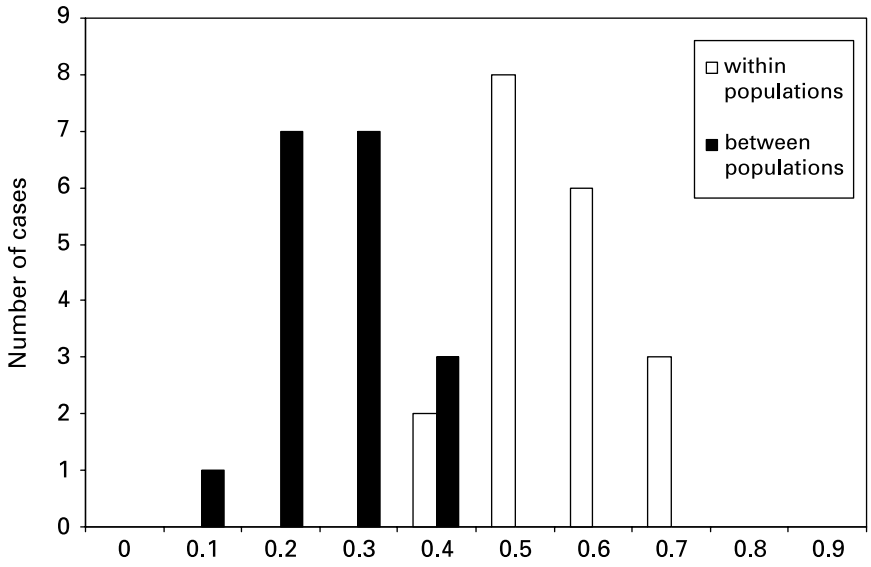

Figure 2. DNA bandsharing coefficients within and between the two populations of Seychelles White-eye (Conception/Mahé).

wings (unpubl. data). No sex- or age-related plumage differences were detected.

There is a significant, but small (1.7%), difference between the average wing length of male and female Seychelles White-eyes although the degree of overlap makes it impossible to distinguish the sexes by this. The sex ratio of the samples from Mahé and Conception did not differ significantly from each other and was not therefore responsible for the average differences measured between the two islands.

The large skew in sex ratio observed on both Mahé and Conception is probably due to a trapping bias. Almost all birds that were sampled were caught by tape luring, to which males are more likely to respond than females. However, there may also be a real deficit of females, as observed in many critically endangered species including Seychelles Magpie-Robin, *Copsychus sechellarum* (Millett *et al.* 1999), Mauritius Pigeon *Columba mayeri* (C. Jones & B. Burn pers. comm.), Echo Parakeet *Psittacula eques echo* (Diamond 1987), and Black Robin *Petroica traversi* (Butler & Merton 1992).

Population genetics

Although the mean number of scorable bands was significantly less on Mahé than on Conception, within-population bandsharing did not differ significantly between islands. Both populations had high within-population bandsharing coefficients (0.59 and 0.61, respectively) compared with levels generally reported for outbred avian populations (range 0.20–0.30, Burke & Bruford 1987, Westneat 1990). These high within-population bandsharing coefficients are comparable with those found in a number of other inbred island populations (see Table 5).

Table 5. Comparisons of within- and between-population bandsharing coefficients (BSC) for a range of endangered bird species.

Species	Authors	Within-population BSC	Between-population BSC
Seychelles White-eye *Zosterops modestus*	This study	0.59–0.61	0.72
Seychelles Warbler *Acrocephalus sechellensis*	Kappa (1998)	0.46–0.52	0.97
Australian Silvereye *Zosterops lateralis chlorocephala*	Degnan (1993)	0.31–0.67	0.99
Chlorocephala vs. *familiaris* race			0.26
Brown Skua *Catharacta lonnbergi*	Millar *et al.* (1994)	0.77–0.76	na
Black Robin *Petroica traversi*	Ardern and Lambert (1997)	0.84	na
New Zealand Bush Robin *Petroica australis*	Ardern and Lambert (1997)	0.45–0.61	na
Pukeko *Porphyrio porphyrio melanotus*	Lambert *et al.* (1994)	0.60–0.40	na

The results indicate that Mahé and Conception both contain low levels of genetic variability. This could be attributed to one or a combination of the following factors: (1) recent founding, (2) small population sizes, and (3) geographical isolation and consequently low, or no, migration between populations (reviewed in Lande 1999). Indeed, there is good historical evidence that the White-eye underwent a severe genetic bottleneck during the first half of the century on Mahé (Crook 1961, Loustau-Lalanne 1961, 1962) and both populations remain very restricted in size and could be considered still to be undergoing population bottlenecks. The Mahé population is estimated at *c.* 50 individuals while Conception may contain up to 330 individuals. However, the effective sizes of these populations are likely to be much lower due to the co-operative breeding system that appears to exist on both islands (Grieg-Smith 1979, Rocamora & François 2000). The effective population size can only be calculated accurately if DNA parentage studies are used to determine the genetic mating system of this species.

The Mahé and Conception populations are genetically differentiated, with a significantly lower mean bandsharing between (0.32) than within populations (0.60). This indicates that the populations are isolated from each other, as only one or two migrants between the two populations per generation would be sufficient to prevent this differentiation (Lande 1999). The similarity of bandsharing between populations corrected for within-population similarity was 0.72, lower than the 0.97 observed between the original Seychelles Warbler population and a daughter population, established on another island in 1988 (Kappa 1998). In a study of Australian Silvereyes *Zosterops lateralis* (Degnan 1993) a similarity of 0.99 was observed between populations within the Capricorn Islands (race *chlorocephala*). But when the Capricorn group was compared to the mainland race (*familiaris*) the similarity index was just 0.26 (see Table 5). However, caution must be taken when interpreting the differences between the present study and that on the Australian Silvereyes as our study comprises only one between-species comparison.

Evolutionarily significant units (ESUs) can be defined as populations that are reproductively isolated over an evolutionarily significant timescale and are evolving independently of other populations (Moritz 1994). The concept of identifying such ESUs using neutral molecular marker data has been the focus of much debate (e.g. Hedrick *et al.* 2001, reviewed in Crandall *et al.* 2000, Pearman 2001). The DNA fingerprinting techniques used in the present study mainly reflect recent demography more than evolutionary differentiation and may therefore be inadequate to separate evolutionary significant units.

Our results suggest that, while the Seychelles White-eye populations are genetically differentiated, the level of similarity between populations (0.32 when no correction for within-population similarity is applied) is only slightly higher than that normally found within outbred avian species (range 0.20–0.30, Burke & Bruford 1987, Westneat 1990) and may not be as extreme as that found between different races of a species. This shallow level of genetic differentiation over a short evolutionary timescale indicates that the two populations could be considered as separate management units but not as separate ESUs (Moritz 1994).

Higher levels of interpopulation bandsharing would be expected if one population had recently (since the near extinction) founded the other. Furthermore, one of the populations should have a higher within-population bandsharing than the other as a result of undergoing both the original bottleneck and a subsequent one as the new population was founded. As this is not the case, the two populations may have separated before the species declined and each contains different fragments from the genome of the original healthy population.

The populations may have separated at any time since the end of the last glaciation 10 000–12 000 years BP, when sea-level rise divided the islands. However it is likely that, before the arrival of humans, White-eyes were plentiful and covered all of Mahé and Conception (and other satellite islands). White-eyes are strong flyers (G.J. Rocamora pers. obs.) and the short distance between Mahé and Conception (1.6 km) would probably not prevent migration and gene exchange between the populations. It appears likely that the White-eye populations have been isolated since anthropogenic disturbance reduced the population on Mahé 100–200 years ago. This concurs with the results of the Silvereye study (Degnan 1993) where two populations, separated by a maximum of 3000–4000 years, showed a much higher degree of genetic differentiation than in this study (see above).

There are some ecological differences between the two populations of Seychelles White-eyes. On Mahé, White-eyes are flexible in habitat preference. Most remaining territories are in residential areas, clearings or forest edges with small-scale agriculture, but some can also be found in tall forests of

introduced Sandragon *Pterocarpus indicus*, or Albizzia *Paraserianthes falcataria* (Watson 1984, Rocamora 1997a, 1997b). In all these places, non-native plants largely dominate the vegetation, which suggests that the species is very adaptable (Mellanby *et al.* 1996; Rocamora & François 2000). On Conception, breeding territories occur throughout the dense mixed woodland found on the island, dominated by Cinnamon C*innamomum verum* (exotic), Takamaka C*alophyllum inophyllum* (native) and Cashew *Anacardium occidentale* (exotic), with abundant indigenous fruiting trees providing berries and seeds for the White-eyes.

Differences have also been found with regard to breeding biology. Birds on Conception are co-operative breeders with up to nine birds contributing to the same nest, which may contain up to seven eggs (Rocamora & François 2000). The composition of nesting groups is unstable, as adult birds contributing to a particular nest are often observed on nests from other neighbouring breeding groups during the same season. Such a complex social breeding system has never been reported from Mahé (Feare 1975, Grieg-Smith 1979, Rocamora 1997b) where only two eggs per nest and smaller co-operative breeding groups (up to five) have been observed. These differences may be due to the extreme scarcity of the species on Mahé, but could also be an adaptation to the high density and saturated environment faced by White-eyes on Conception.

In conclusion, the limited morphological and genetic differentiation found between the two populations, combined with our knowledge of the species' former distribution and history, indicate that the two populations studied are reproductively isolated, probably as a consequence of recent anthropogenic disturbance, and should not, in our view, be considered as two separate races or evolutionary significant units (Crandall *et al.* 2000). However, the observed differences in morphology, ecology and breeding behaviour suggest that each may be locally adapted to its particular environment.

Conservation implications

The most important conclusion from this study, in terms of population management, is that both populations are important genetic reservoirs, and equally deserve to be safeguarded. Consequently, the Species Action Plan 2001–06 (Rocamora & Henriette 2002), which includes transfers of individuals from each population to suitable islands, suggests that each population should be treated as a separate management unit but not as an evolutionarily significant unit. Birds of Conception and Mahé origin may therefore be mixed in one of these island transfers. Since the two populations probably retain different genetic fragments of the original White-eye population, recombining this genetic variation may be beneficial for the species in a novel environment.

Increasing genetic variation has been shown to have positive effects on small, isolated, inbred populations with low genetic variability, e.g. Greater Prairie Chickens *Tympanuchus cupido pinnatus* (Westemeier *et al.* 1998), the Topminnow *Poeciliopsis monacha* (reviewed in Vrijenhoek 1994), *Vipera berus* (Madsen *et al.* 1999), and the method has been assessed for use as a conservation measure in the Florida Panther *Felis concolor coryi* (Hedrick 1995). Benefits include a better capacity to adapt to new environmental constraints (of particular importance for translocated individuals) and reduced vulnerability to extinction due to the negative effects of inbreeding.

If the mixing of the Seychelles White-eye populations on a new island proved successful, with no negative fitness consequences and with both populations interbreeding and contributing towards the genetic variation of the population, then these White-eyes would be used for future transfers to new islands. However, because gene flow may also reduce population fitness within locally well-adapted populations (reviewed in Storfer 1999) the mixing of the two original populations is not recommended on either Conception or Mahé.

This study illustrates the importance of investigating differentiation between remnant populations of a threatened species as a source of information to orientate future conservation and management measures. The Seychelles Fody *Foudia sechellarum*, another threatened species reduced to just four small populations, warrants similar study.

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