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Genetic variation in two endangered *Acrocephalus* species compared to a widespread congener: estimates based on functional and random loci

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Abstract

Substantial genetic variation is hypothesised to be necessary for the long-term survival of species. Therefore, a major aim in conservation is to maintain and restore variation in small and endangered populations. However, in most populations the amount of genetic variation and, thus, the potential threats posed by limited variation are unknown. In the present study, we assess genetic variation, both at 10 microsatellite loci and at the major histocompatibility complex (MHC), in three closely related *Acrocephalus* warbler species with contrasting demographies. We found that the recently bottlenecked, island endemic, Seychelles warbler (*A. sechellensis*; SW) has substantially reduced microsatellite and MHC diversity compared to the widespread great reed warbler (*A. arundinaceus*; GRW). In contrast, another endangered species with a small breeding range, the Basra reed warbler (*A. griseldis*; BRW), harboured as much variation as did the GRW. This suggests that significant genetic variation and, therefore, adaptive potential, remains in the BRW – a situation that should hold as long as its habitat and numbers are maintained. Our study is one of the first to assess genetic variation at both ‘non-critical’ microsatellite markers and ‘critical’ MHC loci within endangered species. The two types of loci provided a similar picture of the genetic variation in the species we studied, but this has not been the case in studies of some other species. Using a combination of specific functional loci and genome-wide random markers appears to be the best way to assess the threat posed by reduced genetic diversity.

INTRODUCTION

Substantial genetic variation is generally hypothesised to be necessary for the long-term survival of populations and species (Frankham, Ballou & Briscoe, 2002). When such variation is present, populations are thought to be better able to adapt to future environmental changes and cope with novel pathogens. Furthermore, genetically depauperate populations may suffer from inbreeding depression, which can contribute towards driving populations extinct (Saccheri *et al.*, 1998; Westemeier *et al.*, 1998; Madsen *et al.*, 1999). Admittedly, there are studies showing that some populations manage to survive over long periods of time despite low levels of genetic variation (Groombridge *et al.*, 2000; Visscher *et al.*, 2001). Nevertheless, the long-term risks posed by low genetic variation have made the management and restoration of genetic variation a major aim in conservation (Frankham *et al.*, 2002).

Small populations are predicted to lose genetic variation due to genetic drift (Nei, Maruyama & Chakraborty, 1975; Chakraborty & Nei, 1977; Wright, 1977; Lande, 1988). In populations that fluctuate over time, the loss of genetic variation accelerates during pronounced and prolonged bottlenecks. The amount of genetic variation is also affected by other parameters, such as selection. For expressed genes, the presence of balancing selection, or negative frequency-dependent selection, will counteract drift-dependent loss of genetic variation, whereas strong directional selection will erode variation by pushing advantageous gene variants to fixation (Hughes & Yeager, 1998; Lynch & Walsh, 1998). The complexity of these demographic and selective processes makes it difficult to predict the degree of genetic variation in most populations and species. Even the basic prediction that very small and restricted populations will have lower levels of genetic variation than large ones (Frankham, 1996) has sometimes been refuted. In studies that have contrasted the amount of genetic variation in endangered and widespread congeners, some have found comparatively low levels of genetic variation in the rare species (e.g. Godt, Walker & Hamrick, 1997; Groombridge *et al.*, 2000; Ujvari *et al.*, 2002), whereas others have found similar levels (e.g.

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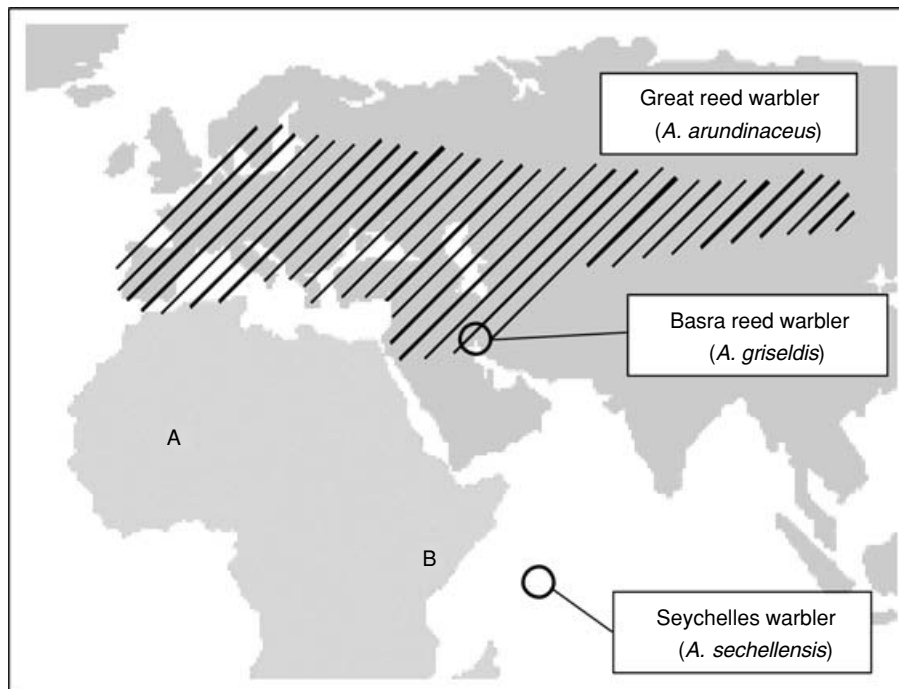


Fig. 1. Current distribution ranges of two endangered *Acrocephalus* species, the Basra reed warbler and the Seychelles warbler (circles), and their widespread congener, the great reed warbler (lines). Also indicated are the sites where the great reed warblers (A) and the Basra reed warblers (B) were sampled (see Methods).

Lewis & Crawford, 1995; Ge *et al.*, 1999; Madsen *et al.*, 2000).

The fact that some studies find the expected association between population size and genetic variation while others do not, may be due to the choice of loci used to assess genetic variation (Frankham *et al.*, 2002). This is demonstrated clearly in studies of single data sets analysed using multiple types of loci (Yan *et al.*, 1999; Madsen *et al.*, 2000) and is likely to reflect the different mutational-drift equilibria and patterns of selection at different loci (Frankham, 1996; Hedrick, 1999; Frankham *et al.*, 2002). Consequently, care should be taken over selecting which loci to base assessments of diversity upon, especially when the results may have important ramifications for the conservation of a species (Reed & Frankham, 2001, 2003; van Tienderen *et al.*, 2002).

The endemic Seychelles warbler (*Acrocephalus sechellensis*; SW) was pushed to the verge of extinction by anthropogenic disturbance and in the 1960s less than 30 individuals remained on a single island (Crook, 1960). Careful management of the species and its habitat has restored the SW population to a total of *ca.* 2000 birds on four islands (Komdeur, 1994, 2003; Richardson, 2001). The Basra reed warbler (*A. griseldis*; BRW) is restricted to wetlands within a limited area of southern Iraq and Kuwait (Cramp, 1992). Information on the BRW is scarce (Cramp 1992; Pearson & Backhurst, 1988; Tyler *et al.*, 1997), but the most up-to-date source estimates its population size at a few thousand pairs (BirdLife International; <http://www.birdlife.net/print.html>). In contrast to the aforementioned rare species, the great reed warbler (*A. arundinaceus*; GRW) breeds over large parts of Eurasia (Catchpole, Leisler & Winkler, 1985; Cramp, 1992; Hansson,

Gavrilov & Gavrilov, 2003), with population estimates of over one million pairs (Cramp, 1992; Hagemeyer & Blair, 1997).

In the present study, we compare the genetic variation of these three closely related species of *Acrocephalus*. We aim to evaluate the correspondence between population size and genetic variation in the species and to determine the genetic status of the endangered BRWs and SWs. We assess genetic variation at random and functional loci by analysing both non-coding microsatellite markers (Jarne & Lagoda, 1996) and coding loci in the major histocompatibility complex (MHC) – an area involved in the adaptive immune system of vertebrates (Hughes & Yeager, 1998; Hess & Edwards, 2002). This combination of loci allows us to obtain relevant estimates of the genetic variation of these species (Reed & Frankham, 2001, 2003; van Tienderen *et al.*, 2002).

METHODS

Study species and general methods

The three species included in the present study are closely related and, in phylogenies based on variation at the mitochondrial cytochrome *b* gene, cluster together with the other relatively large and unstreaked *Acrocephalus* spp. (e.g. *A. stentoreus* and *A. gracilirostis*: Helbig & Seibold, 1999). The mitochondrial sequence difference is about 10% for the three species-pairs (GRW versus BRW = 9.7%; GRW versus SW = 8.5%; BRW versus SW = 10.5%: Helbig & Seibold, 1999).

The GRW breeds in the reed–water interface of wetlands over large parts of Europe and Asia (Fig. 1;

Catchpole *et al.*, 1985; Cramp, 1992; Hansson *et al.*, 2003) but overwinters in sub-Saharan Africa (Bensch *et al.*, 1991; Cramp, 1992). Two subspecies are recognised, *A. a. arundinaceus* in the western part of the range (Europe) and *A. a. zarudnyi* in the eastern part (Asia), respectively (Cramp, 1992). The 22 individuals in the present study were captured using mist-nests at a stopover site at Malamfatori, close to Lake Chad in north eastern Nigeria, during the birds' migration in August–November 2000 (see Fig. 1; Ottosson, Barlein & Hjort, 2002). These GRWs belonged to the western sub-species *A. a. arundinaceus* and are expected to originate from many different European populations, because (1) the stopover site is along a general migration route for European GRWs as suggested by ringing recoveries and censuses (Bensch *et al.*, 1991; Cramp, 1992; Ottosson *et al.*, 2002) and (2) samples were collected throughout the migratory period. Thus, the sample of *A. a. arundinaceus* should contain little bias with regard to the population sub-structuring known to occur within Europe (Bensch & Hasselquist, 1999; Hansson *et al.*, 2000; Hansson, 2003) and is likely to be a much better estimate of the species' total genetic variation than a sample of breeding birds from one or a few breeding localities.

The BRW is endemic to the Mesopotamian marshes in southern Iraq and parts of Kuwait (Fig. 1; Cramp, 1992). The species' breeding ecology is described as being similar to that of the GRW (Cramp, 1992), but the species is under-researched and many details regarding its ecology are still unknown. The species migrates to central eastern Africa (Pearson & Backhurst, 1988; Cramp, 1992; Tyler *et al.*, 1997) and during the last three decades about 60 (range 3–196) BRWs have been captured annually at Ngulia Ringing Station, Tsavo West National Park, south eastern Kenya (Pearson & Backhurst, 1988; G. Backhurst, pers. com.; Fig. 1). Of the birds included in the present study, five were examined at Ngulia in November–December 1990 and 17 in November–December 1998. Migrating GRW and BRW are easily separated from each other and from all other *Acrocephalus* spp., by means of a few morphological measurements (length of bill, head, wing and tail, and body mass: Pearson & Backhurst, 1988; Cramp, 1992; Hansson *et al.*, 2003).

The SW is sedentary and endemic to evergreen forests on the Seychelles islands (Komdeur, 1992). Between 1959 and 1968, this species went through a severe genetic bottleneck with only 26–29 birds remaining on the island of Cousin (Crook, 1960). Since then the population has increased to approximately 2000 individuals in four island populations (D. S. Richardson, unpublished results). The 22 individuals in the present study were caught on Cousin in 2002, well after the population increase and, on the basis of pedigree and molecular data, are not close relatives (Richardson *et al.*, 2001; Richardson, Burke & Komdeur, 2002).

A small amount of blood (< 25 µl) was taken from the brachial vein of each bird. Blood samples were stored in either SET buffer or ethanol and DNA was extracted using either phenol/chloroform-isoamylalcohol (Bruford *et al.*, 1998) or a simplified salt extraction method (Richardson *et al.*, 2001).

Microsatellite analyses

We used the following procedure to select microsatellite loci. From a database of 50 microsatellite loci developed in the Seychelles warbler (Richardson *et al.*, 2000), we selected 10 markers that were polymorphic in the marsh warbler, *A. palustris* (Richardson *et al.*, 2000), an outgroup species (Helbig & Seibold, 1999). By selecting loci that were polymorphic in this species, we reduced the potential problems of amplification failure due to mutations at the primer sites (Primmer, Møller & Ellegren, 1996) and of low levels of polymorphism due to low mutation rates caused by few repeat units (Ellegren, Primmer & Sheldon, 1995), in the species for which the primers were not originally designed. Detailed information about the microsatellite loci, including specific PCR conditions, can be found in Richardson *et al.* (2000).

Amplified microsatellite alleles were separated using electrophoresis in acrylamide gels. Gels were scanned in a FluorImage SI (Molecular Dynamics Inc.) to visualise the fluorescein-labelled primers and polymerase chain reaction (PCR)-products. In every gel, two reference individuals (two GRWs) were run as size standards. B. H. scored all microsatellite gels. Allele frequencies, allele counts, heterozygosity calculations, estimates of null allele frequencies and tests of Hardy–Weinberg equilibrium were evaluated for each species separately using the program CERVUS 2.0 (Marshall *et al.*, 1998).

MHC analyses

To study genetic variation at the MHC, we used motif-specific primer combinations that amplify exon 3 sequences from one or several genes in the class I gene complex (Westerdahl, Witzell & von Schantz, 1999; Richardson & Westerdahl, 2003; Westerdahl *et al.*, 2003). We chose the exon 3 because it encodes the peptide-binding region of the MHC class I genes and, therefore, is crucial for antigen recognition and adaptive immunity (Hughes & Yeager, 1998). The exon 3 consists of 274 base-pairs (bp), of which we amplified 260 bp (Westerdahl *et al.*, 1999, 2003).

The PCR products were separated using denaturing acrylamide/bisacrylamide gels following the denaturing gradient gel electrophoresis technique (DGGE; for details see Richardson & Westerdahl, 2003; Westerdahl *et al.*, 2003). Gels were stained with SYBR gold (Molecular Probes) and the alleles were visualised in a FluorImage SI (Molecular Dynamics Inc.). The screening method is repeatable and gives an estimate of the MHC class I exon 3 variation within each individual. A similar method has been used when studying MHC polymorphism in three-spined stickleback (*Gasterosteus aculeatus*: Reusch *et al.*, 2001; Wegner, Reusch & Kalbe, 2003). The primers were originally developed for use in the GRW, but amplify the same length and region of the exon 3 in the SW (as determined by sequence similarity; D.S.R., unpublished results). The method worked equally well on the BRW, as well as on the marsh warbler, and there is no reason to think that it is not amplifying the same exon 3 region in these closely related species.

Table 1. Number of alleles (k) and genotyped individuals (N), and expected and observed heterozygosity (H_E and H_O , respectively), at 10 microsatellite loci in three warblers of the genus *Acrocephalus*

Locus	Great reed warbler				Basra reed warbler				Seychelles warbler			
	k	N	H_E	H_O	k	N	H_E	H_O	k	N	H_E	H_O
Ase2	1	22	0.00	0.00	3	22	0.53	0.50	3	22	0.51	0.46
Ase8	3	22	0.45	0.36	10	22	0.90	0.77	1	22	0.00	0.00
Ase9	12	22	0.78	0.73	6	22	0.73	0.68	3	22	0.52	0.64
Ase11	9	22	0.85	0.73	11	21	0.88	0.86	3	22	0.56	0.46
Ase12	3	22	0.29	0.23	6	22	0.75	0.73	1	22	0.00	0.00
Ase19	1	22	0.00	0.00	3	22	0.42	0.36	2	22	0.50	0.77
Ase27	32	22	0.99	1.00	22	22	0.96	0.96	4	22	0.57	0.55
Ase34	5	22	0.68	0.73	5	22	0.57	0.46	1	21	0.00	0.00
Ase56	7	22	0.40	0.46	14	22	0.92	1.00	2	22	0.30	0.36
Ase58	19	21	0.92	0.86	25	21	0.97	0.86	5	20	0.71	0.85

Individuals of the same species were run on the same gel so that bandsharing between adjacent dyads of individuals could be compared. Each individual was compared to the individual directly to the left of it on the gel. D.S.R. scored all MHC gels. The number of DGGE bands present in each individual was counted and a bandsharing coefficient between dyads was calculated as described by Bruford *et al.* (1998).

Statistical analyses

We used number of alleles and expected heterozygosity to measure microsatellite diversity in our analyses, because these two measures, although expected to be correlated, describe allelic data somewhat differently: number of alleles is affected by the presence of both common and rare alleles, whereas expected heterozygosity is influenced mainly by the allele frequencies of common alleles. These are two of the most commonly used measures to describe allelic diversity in population genetic studies (Frankham *et al.*, 2002). Similarly, we used number of bands and bandsharing coefficient to measure MHC diversity. We analysed the data using two-tailed non-parametric tests: Wilcoxon signed rank tests (denoted z ; see below) for the microsatellites because this allowed inter-specific tests paired for loci and Mann-Whitney U -test/Kruskal–Wallis tests (denoted U and H , respectively; see below) for the MHC data because this allowed tests of non-normally distributed samples.

RESULTS

Microsatellite loci

The microsatellite primers amplified products in all species and almost all PCRs were successful (654 out of 660 PCRs: Table 1). The BRW was polymorphic at all 10 loci, whereas the GRW and the SW were polymorphic at 8 and 7 loci, respectively. For all species, and for all loci, observed heterozygosity was similar to expected heterozygosity (Table 1) and in the few cases where a test was possible, we found no significant deviation

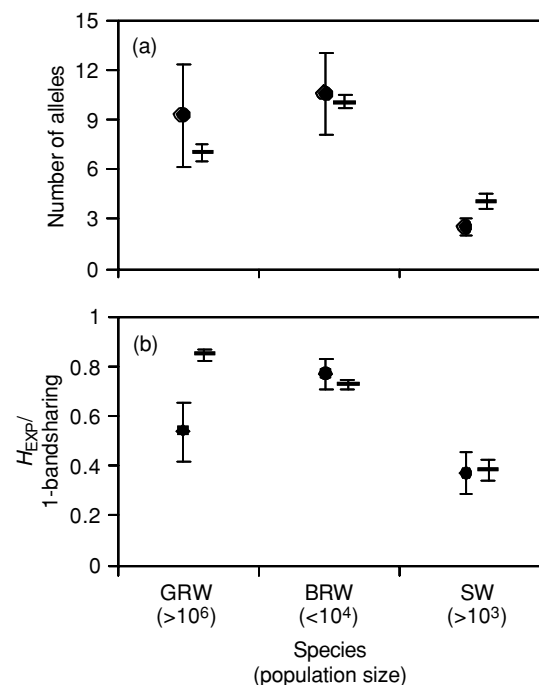


Fig. 2. Mean \pm standard error (s.e.) of (a) number of microsatellite (filled circles) and MHC alleles (lines) and (b) expected microsatellite heterozygosity (filled circles) and MHC bandsharing coefficients (given as 1-bandsharing: lines), in great reed warblers (GRW), Basra reed warblers (BRW) and Seychelles warblers (SW). Also shown is the estimated current population size.

from Hardy–Weinberg equilibrium (BRW at Ase2, SW at Ase27: $\chi^2 \leq 0.06$, d.f. = 1, NS). Estimates of null allele frequency ranged between 0.20 (BRW at Ase8) and -0.23 (SW at Ase19).

The mean number of microsatellite alleles was substantially lower for the SW than for the other two species (GRW: $z = -0.93$, $n = 10$ loci, $P = 0.016$; BRW: $z = -2.67$, $n = 10$ loci, $P = 0.008$; see Fig. 2 for mean \pm standard error (s.e.) number of alleles per locus). There was no significant difference in number of alleles between the GRW and the BRW ($z = -0.95$, $n = 10$ loci, $P = 0.34$). A Fisher combined test (Sokal & Rohlf, 1995)

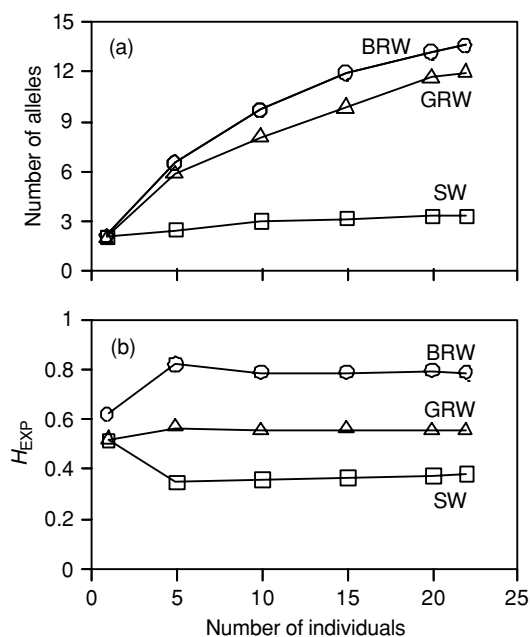


Fig. 3. Mean number of alleles (a) and mean expected heterozygosity (b) at the 10 microsatellite loci for different numbers of great reed warblers (GRW), Basra reed warblers (BRW) and Seychelles warblers (SW).

of the P -values from the pair-wise species tests showed that the difference in number of alleles was significant on the level of all species ($S_1 = -2 \times \Sigma(\log_e(P_i)) = 20.1$, d.f. = 1, $P < 0.005$).

Slightly different patterns were found using expected heterozygosity (see Fig. 2 for mean \pm s.e. of expected heterozygosity per locus). The tests showed that the heterozygosity of the BRW was significantly higher than that of the SW ($z = 2.60$, $n = 10$ loci, $P = 0.009$), but not significantly different from that of the GRW ($z = 1.78$, $n = 10$ loci, $P = 0.074$). Despite the relatively large numerical difference between the GRW and SW in expected heterozygosity, this difference was also non-significant ($z = 1.07$, $n = 10$ loci, $P = 0.29$). The variation in expected heterozygosity differed significantly on the level of all species ($S_1 = 17.1$, d.f. = 1, $P < 0.01$).

Our analyses are based on genotype data from 22 individuals of each species. To evaluate whether this sample size was large enough to estimate the number of alleles and expected heterozygosity, we plotted the values of these parameters at different numbers of scored individuals (Fig. 3). These graphs showed that the asymptote value of mean number of alleles was not yet reached in the samples of the GRW and BRW, whereas the asymptote was reached in the SW (Fig. 3). Thus, more GRW and BRW alleles would have been detected had more individuals been genotyped. The graphs suggest, however, that the main results – i.e. that the mean number of microsatellite alleles was substantially lower in SW than in the other two species, and that GRW and BRW had a similar number of alleles – would have been unaffected by including more individuals in the analyses. Mean expected heterozygosity reached stable values in all three

species after approximately 10 genotyped individuals (Fig. 3).

The MHC loci

MHC alleles were amplified in 65 out of the 66 individuals. The number of different alleles varied significantly between species ($H = 41.96$, d.f. = 2, $P < 0.001$), and was lowest in the SW and highest in the BRW (Fig. 2). When species-pairs were compared, the difference in number of MHC alleles was significant in all three pair-wise comparisons (GRW versus BRW, $U_{21,22} = 66.00$, $P < 0.001$; GRW versus SW, $U_{21,22} = 61.00$, $P < 0.001$; BRW versus SW, $U_{22,22} = 5.50$, $P < 0.001$). Also, the bandsharing coefficients differed significantly between species ($H = 41.28$, d.f. = 2, $P < 0.001$; Fig. 2). Here, the BRW scored higher than the GRW ($U_{20,22} = 101.00$, $P = 0.004$), whereas the SW scored higher than both the other species (GRW, $U_{20,22} = 4.00$, $P < 0.001$; BRW, $U_{22,22} = 16.00$, $P < 0.001$).

DISCUSSION

Our results show that the GRW and the BRW populations contain similar high levels of genetic variation, whereas the SW population is genetically less variable. This pattern was statistically true for the comparison of number of microsatellite alleles. For microsatellite heterozygosity, the analyses indicated that the GRW tended to be less heterozygous than the BRW and although the SW appeared to have lower levels than the GRW, this difference was not significant. It is possible that the few monomorphic loci in the GRW and the SW caused large variation in the heterozygosity parameter, resulting in low power in these comparisons. That the BRW had more MHC alleles but still higher bandsharing than the GRW is intriguing. A possible explanation is that balancing selection, which appears to play a determinant role in MHC evolution (Hughes & Yeager, 1998; Hess & Edwards, 2002), is maintaining both the number and the diversity of MHC alleles in the BRW. Since all individuals from the BRW are from one population in a limited geographical area, they all face the same selection pressures maintaining the same combinations of MHC alleles, thus individuals can have a high number of alleles but still be similar to each other. In contrast, the GRW's massive pan-European population has significant population sub-structure (Bensch & Hasselquist, 1999; Hansson *et al.*, 2000; Hansson, 2003). Therefore the same process, where similar selection pressures are occurring on all birds, is unlikely in GRWs and genetic differences between sub-populations may be represented in our data set.

Genetic variation in the SW was about half to one third of that of its congeners. This estimate is in line with previous data from this species (Komdeur, Kappe & van de Zande, 1998; Richardson & Westerdahl, 2003). Similar low levels of variation have been found in populations of some other endemic island species,

for example, Californian channel island foxes, *Urocyon littoralis* (Gilbert *et al.*, 1990), Laysan finch, *Telespiza cantans* (Tarr, Conant & Fleischer, 1998), Mariana crow, *Corvus kubaryi* (Tarr & Fleischer, 1999) and Seychelles white-eye, *Zosterops modestus* (Rocamora & Richardson, 2003; see also studies reviewed therein). It is plausible that most of the genetic variation was depleted during the recent bottleneck that the SW went through (Komdeur *et al.*, 1998; Richardson & Westerdahl, 2003), but also earlier undocumented bottlenecks are very likely to have occurred in the isolated and fragmented island habitat provided by the Seychelles islands.

The substantial genetic variation at both microsatellites and MHC found in the BRW is important as this suggests that the species retains biodiversity and high adaptive potential (c.f. Hughes & Yeager, 1998; Hess & Edwards, 2002; Reed & Frankham, 2003). This positive situation should hold as long as the habitat, and thus the population, remains intact. Unfortunately, the Mesopotamian marshes where the species breeds have been almost entirely drained during the last decade and numbers are currently declining (see BirdLife International, <http://www.birdlife.net/print.html>). If this decline continues, the expression of genetic load could have disastrous reproductive consequences for this species (c.f. Saccheri *et al.*, 1998; Westemeier *et al.*, 1998; Madsen *et al.*, 1999). Updated population estimates and data on habitat utilisation at the breeding quarters are necessary to assess the long-term survival status of the BRW and new genetic surveys on this species may be required in the near future.

The results from our study indicate that regardless of which loci are used, and contrary to predictions (Frankham, 1996), genetic variation does not always correlate strongly with population size. Other studies on a range of species have shown similar results (e.g. Lewis & Crawford, 1995; Ge *et al.*, 1999; Madsen *et al.*, 2000). This lack of correlation may be a result of the complicated processes involved in determining genetic variation at specific loci (see Introduction, above), or because our sample of migrating GRWs represented populations from only a part of the European breeding range (thus, should be associated with a somewhat smaller population size; see Methods, above). However, in our study the fact that the widely distributed GRW showed no more genetic variation than the much rarer BRW, could indicate that the current GRW population size provides a very poor estimate of the species' long-term effective population size, which is the relevant parameter in this context (Wright, 1977; Lande, 1988). In line with this reasoning, mitochondrial DNA analyses show that the long-term effective population size of the GRW is in fact only in the order of 1% of the current population size (Bensch & Hasselquist, 1999). Alternatively, it might be suggested that ongoing hybridisation and gene flow between the BRW and other species, e.g. the GRW, may have caused the relatively high level of variation in the BRW. Indeed, related *Acrocephalus* sp. may occasionally hybridise (Hansson *et al.*, 2003; Hansson, Roggeman & De Smet, 2004; c.f. Grant & Grant

1992). However, this is not a likely explanation for our results. The mitochondrial sequence divergence between BRWs and GRWs is about 10% (Helbig & Seibold, 1999), which implies that they have been separated for a very long period of time. Moreover, these two species had completely non-overlapping microsatellite allele frequencies at locus Ase12 and very different allele frequency distributions at most other loci (data not shown), which suggest that there is no present gene flow between them.

In our study the 'critical' MHC loci and the 'non-critical' microsatellite markers provide a similar picture of genetic variation in the species, i.e. diversity is similar in the GRW and the BRW but reduced in the SW. While our results from different types of loci support each other, we know that this is not always the case (e.g. Yan *et al.*, 1999; Madsen *et al.*, 2000). To date, most biodiversity studies have focused on allozymes or non-coding genetic markers (for a review, see Frankham *et al.*, 2002). However, recent studies have criticised the use of such markers as they may not reflect the variation that is important to the fitness of the species in question (Reed & Frankham, 2001; van Tienderen *et al.*, 2002; Bekessy *et al.*, 2003; but see Reed & Frankham, 2003). Still, as pointed out by van Tienderen *et al.* (2002), replacing neutral markers, which cover a large part of the genome, with markers that only target a small subset of genes, is risky when assessing the biodiversity of endangered species, especially if the threat to the species is from genome-wide inbreeding depression. Using a combination of genome-wide neutral markers and specific 'critical' loci (chosen to measure variability at ecologically important traits) appears to be the safest way to assess the threat posed by reduced genetic diversity in a species.

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