doi:10.1111/j.1558-5646.2011.01397.x

A TIGHT BALANCE BETWEEN NATURAL SELECTION AND GENE FLOW IN A SOUTHERN AFRICAN ARID-ZONE ENDEMIC BIRD

Ângela M. Ribeiro,[1,2,3] Penn Lloyd[2] and Rauri C. K. Bowie[1,2]

[1]*Department of Integrative Biology and Museum of Vertebrate Zoology, University of California, 3101 Valley Life Science Building, Berkeley, California 94720*

[2]*Percy FitzPatrick Institute, DST/NRF Centre of Excellence, University of Cape Town, Rondebosch 7701, Cape Town, South Africa*

[3]*Email: ribeiro.angela@gmail.com*

Received December 12, 2010
Accepted June 21, 2011
Data Archived: Dryad doi:10.5061/dryad.0pm3v

Gene flow is traditionally thought to be antagonistic to population differentiation and local adaptation. However, recent studies have demonstrated that local adaptation can proceed provided that selection is greater than the homogenizing effects of gene flow. We extend these initial studies by combining ecology (climate), phenotype (body size), physiological genetics (oxidative phosphorylation genes), and neutral loci (nuclear microsatellites and introns) to test whether selection can counter-balance gene flow and hence promote local adaptation in a bird whose distribution spans an aridity gradient. Our results show that the Karoo scrub-robin's climatic niche is spatially structured, providing the potential for local adaptation to develop. We found remarkably discordant patterns of divergence among mtDNA, morphology, and neutral loci. For the mitochondrial genes, two amino acid replacements, strong population structure and reduced gene flow were associated with the environmental gradient separating western coastal sites from the interior of southern Africa. In contrast, morphology and the neutral loci exhibited variation independent of environmental variables, and revealed extensive levels of gene flow across the aridity gradient, 50 times larger than the estimates for mitochondrial genes. Together, our results suggest that selective pressures on physiology, mediated by the mitochondrial genome, may well be a common mechanism for facilitating local adaptation to new climatic conditions.

KEY WORDS: **climatic envelope, morphology, mtDNA OXPHOS genes, neutral loci, Passeriformes.**

Nearly a century ago, Joseph Grinnell (1924) underscored the importance of environmental-based selection in maintaining species adapted to their environments: "The only mechanism of which I have ever heard, that I can reasonably conceive as operating to permit of the molding of species under the stress of environments is natural selection." Grinnell's verbal argument was later formalized to set the framework for subsequent research about the role of selection in promoting adaptation of populations to local conditions. Theoretical models predict that local adaptation can occur provided that selection is strong relative to migration. Under the migration–selection model (Levene 1953; Bulmer 1972; Endler 1977; Lenomard 2002) two populations (1 and 2) inhabit two environmental patches (X and Y) and exchange m migrants per generation. Alleles A and a have antagonistic fitness, whether they occur in patch X or Y. In this model, migration tends to limit or prevent differentiation among populations whereas natural selection removes less-fit immigrants from the population. Traditionally, restricted gene flow has been seen as a requisite for local adaptation to develop. Nonetheless, theoretical (e.g., Endler 1977; Doebeli and Dieckmann 2003) and empirical evidence (e.g., Smith et al. 2001; Ogden and Thorpe 2002; Mullen and Hoekstra 2008; Nosil et al. 2009) challenge this viewpoint: strong

disruptive selection can maintain adaptation to different environments despite very high levels of gene flow, particularly if selection acts on one or a few traits with large effects on fitness.

Aside from selection, historical demographic processes (e.g., colonization of a new habitat) can also generate variation in allele frequencies and/or phenotypes (Endler 1977). Therefore, comparing loci that track demography (neutral) with presumed adaptive variation (genetic and/or phenotypic) is essential if we are to disentangle the effects of nonadaptive versus adaptive processes and hence gain an understanding of the mechanisms underlying the observed spatial pattern in phenotype and/or genotype across the landscape of interest (e.g., Rosenblum et al. 2007).

Many adaptive responses to local environmental conditions are thought to be mediated by physiological mechanisms (Ricklefs and Wikelski 2002). Compelling examples of local adaptation induced by selective pressures acting on molecular physiology have emerged from studies of Deer mice (Storz et al. 2007), Rufous-collared sparrows (Cheviron and Brumfield 2009), Yellow-bellied pintails (McCracken et al. 2009), and the Bar-headed goose (Scott et al. 2011). Most of these studies have focused on mechanisms that enable individuals to persist along extreme hypoxic gradients. However, the mechanisms that facilitate adaptation to other extreme habitat types remain largely unknown. For instance, daily temperature fluctuations and, the limited and unpredictable availability of water and food in arid environments might affect energy and water requirements in homeotherms (Lillywhite and Navas 2006). Taking advantage of well-developed population genetic theory, we integrate ecology, molecular genetics, and morphology to study how the interplay between natural selection and gene flow affects the spatial structure across the entire range of a bird endemic to the south-western arid-zone of Africa (Fig. 1A).

The Karoo scrub-robin, *Cercotrichas coryphaeus*, is a sedentary, medium-sized, ground-feeding insectivorous bird (18–23 g; Oatley and Arnott 1998) that exhibits female-biased dispersal (Ribeiro et al. 2011). Its geographical range encompasses an area with rainfall and temperature gradients, which result from two main processes: the cold Benguela Current running northward along the western coast of southern Africa and the "rain shadow effect" created by the Drakensberg Mountains in the eastern part of the subcontinent (Werger 1986). Thus, two conceptual axes can be used to characterize the overall climate within the range of the Karoo scrub-robin (Fig. 1A, B): (1) a latitudinal gradient where precipitation decreases northward, from the Cape Province into the southern Namib Desert; and (2) a longitudinal trend in seasonality extending from a winter rainfall regime along the western coast to a summer rainfall regime in the interior, with a narrow intermediate area with a year-round rainfall regime (Desmet and Cowling 1999; Chase and Medows 2007). This peculiar climatic setting was established during the Holocene (Chase and Meadows 2007) and has a dramatic effect on vegetation physiognomy (Fig. 1c), primary productivity, and drinking water availability across the landscape (Dean and Milton 2004). This provides an opportunity for directionality of natural selection to differ across the range and hence to promote adaptation to local conditions.

Should geographically varying selective pressures in the southern African arid-zone suffice to overcome the influx of nonadaptive traits/alleles, we predict (Fig. 2): (1) geographical variation in environmental variables to create a nonrandom distribution of adaptive traits as opposed to neutral traits, which are expected to vary independently of environmental variables, (2) strongly differentiated populations coincident with spatial climatic structure within the range as opposed to a pattern of isolation by distance, which would only depend on effective dispersal distance (gene flow) and effective population size (genetic drift), and (3) contrasting levels of gene flow for adaptive and neutral loci.

Recent studies have shown that a combination of low basal metabolic rate (BMR) and evaporative water loss (EWL) is favored in passerine birds from dry and hot environments (Tieleman et al. 2003; Williams and Tieleman 2005). Therefore, we tested the above predictions by quantifying two types of traits likely to mediate the adaptive response: morphology and physiological genetics. First, birds can reduce their EWL by decreasing the amount of water lost by evaporation through the skin (Williams and Tieleman 2005). One possible mechanism to reduce water loss through the skin is to minimize the surface area exposed. Thus, we examined evidence of a trend toward increased body size in response to greater aridity as a means to minimize cutaneous water loss. Second, mitochondria play a central role in energy and heat production (Hochachka and Somero 2002). Because of the uniparental mode of inheritance of the mitochondrial genome, and the high mutation rate favoring the rapid expression of new advantageous alleles, several authors have proposed that mitochondria are likely to mediate an adaptive response to extreme environmental challenges and dietary conditions (e.g., Mishmar et al. 2003; Ballard and Whitlock 2004; Gershoni et al. 2009; Ballard and Melvin 2010). Furthermore, Tielman et al. (2009) recently demonstrated the functionality of the mitochondrial genome in the regulation of energetic metabolism in birds, particularly BMR. We examined the genetic variability underlying a key enzyme from the oxidative phosphorylation pathway (OXPHOS) with the rationale that climate-based selection might act on amino acid substitutions in the mtDNA and thus impact metabolism.

Methods

FIELD PROCEDURES

Four field expeditions, across different years, were conducted to capture birds from 24 localities in southern Africa using mist-nets and traps (Fig. 1). This sampling scheme encompassed the

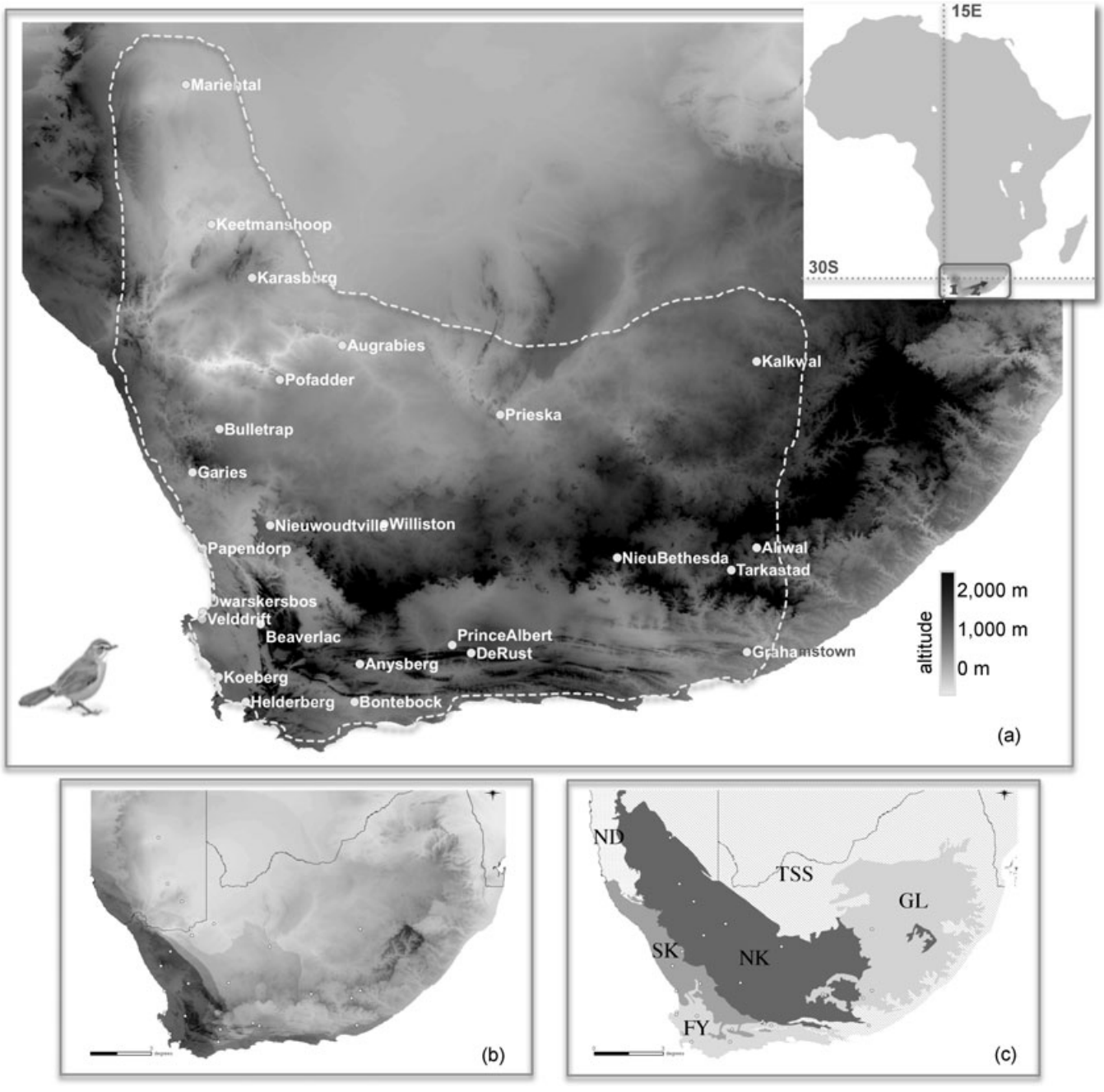

Figure 1. **(A) Geographical range and the 24 sampling localities for the Karoo scrub-robin. Inset showing location in Africa and the two conceptual axes used to characterize climate across the species range. (B) Variation in mean annual temperature over southern Africa represented in shades of gray, lighter shade = larger temperature range. (C) Biomes in Southern Africa: FY = Fynbos, GL = Grassland, ND = Namib Desert, NK = Nama Karoo, SK = Succulent Karoo, TSS = Tree and shrub savanna.**

entire distribution of the species. Captured birds were permanently banded with a uniquely numbered aluminum ring, weighed, measured, and bled by puncture of the subbrachial vein. Alternatively, a representative voucher specimen was collected. Tarsus-length was measured with a digital caliper to an accuracy of 0.1 mm and mass was measured with a 50 g Pesola spring scale to an accuracy of 0.5 g. Juvenile birds were distinguished on the basis of plumage features (Oatley and Arnott 1988)

ENVIRONMENTAL AND MORHOLOGICAL VARIATION

Eighteen bioclimatic variables at a resolution of 30 arc-seconds (1-km^2 grid) were obtained from the WorldClim database (Hijmans et al. 2005). The BIOCLIM algorithm (Nix 1986) as implemented in DIVA-GIS version 5.4 (Hijmans et al. 2004) was used to extract climatic data for each of our geo-referenced localities. Bioclimatic variables were used to characterize the climatic envelope by implementing a PCA. Because principal components

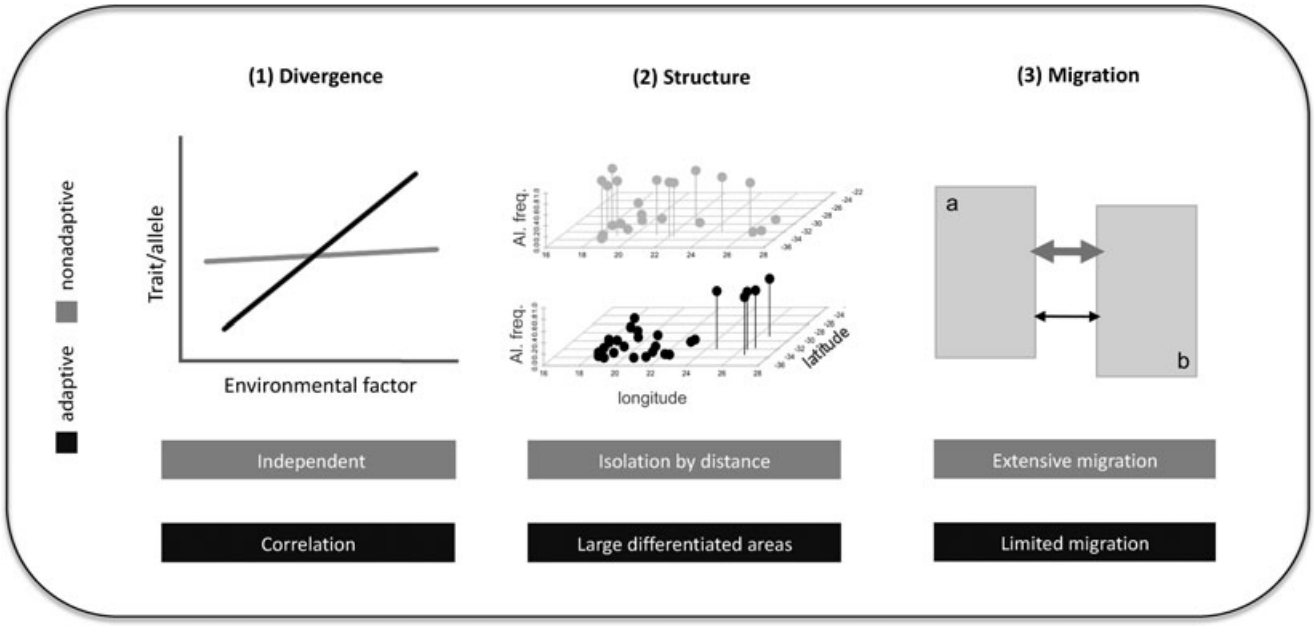

Figure 2. Schematic description of predicted patterns of: (1) divergence, (2) population structure, and (3) migration rates for adaptive and nonadaptive traits/loci under a migration–selection model. (a) and (b) represent environmentally distinct patches. The width of the arrow in (3) is proportional to migration rates.

(PC) are dependent on the units of the original variables, before compressing the data into new uncorrelated variables, the original variables were standardized by the maximum value. The PCs were extracted from a covariance matrix and the scores were used to document patterns of environmental variation across the geographical range of the species, as well as to estimate environmental distances between populations along each resulting climatic vector.

Juvenile birds were excluded from the morphological analyses. Body mass (g) and tarsus-length (mm) of adult birds (n = 270) were used to quantify phenotypic variation across the entire range. To test the effect of climate (two factors: PC1 and PC2) on morphology (response variables: body mass and tarsus-length), we implemented a MANOVA on $\log_{10}$-transformed data that were previously tested for normality. We chose to use body mass and tarsus-length as a proxy for body size, variables that are likely to reflect climate-based selective pressures for reduced water loss. When the overall MANOVA was significant (Wilks' test), we next examined the univariate F-test for each variable to understand its respective effect. When the ANOVA was significant, a post-hoc Tukey's HSD test was used to discriminate which populations occupied significantly different regions of the morphospace. All statistical analyses were performed using R (R Development Core Team 2010).

GENETIC DATA

Genomic DNA was extracted from blood/tissue samples using DNeasy kits (Qiagen, Valencia, CA). The sex of all birds was determined using a PCR-based assay (Fridolfsson and Ellegren 1999). Of the 16 polypeptides that comprise the fifth complex of the OXPHOS pathway, the ATP synthase, we chose to examine the only two polypeptides that are mtDNA-encoded: ATPase subunits 6 and 8. Subunits 6 and 8 play an essential role in the translocation of protons across the inner membrane and in the assembly of the complex, respectively (Pedersen et al. 2000).

DNA sequences

Two mitochondrial protein-coding genes (ATP6 and ATP8), three nuclear autosomal loci (Gapdh-intron11, *Gallus gallus* chromosome 1; βFib-intron5, *G. gallus* chromosome 4; *15691 G. gallus* chromosome 5) and one Z-linked intron (BRM-intron15) were amplified by PCR using the following primers: ATP6–8 (A8L8929 and A6H9929, E. Bermimgham Lab), Gapdh (Friesen et al. 1997), Fib5 (Fuchs et al. 2004; Kimball et al. 2009), *15691* (Backström et al. 2008), and BRM (Goodwin 1997). All reactions were performed in a total volume of 10 μl with 10–20 ng of genomic DNA, 0.5U of Taq Polymerase (Roche), GeneAmp 10× PCR Gold Buffer, 2.0–2.5 mM $MgCl_2$, 0.3 mM of each dNTP, and primer concentrations of 0.15 mM. The thermocycling profile consisted of an initial denaturizing step at 95°C for 3 min followed by 35 cycles at 95°C for 30 s, and locus-specific annealing temperature (55–60°C) for 30 s, and 72°C for 30 s, with a final extension step at 72°C for 7 min. Cycle-sequencing reactions were performed in both forward and reverse directions using the ABI BigDye Terminator Kit version 3.1 (Applied Biosystems, Foster City, CA). Cycle sequencing reaction products were

purified using Sephadex columns and then analyzed on an ABI 3730 automated sequencer (Applied Biosystems).

Sequences were edited and aligned using CodonCode Aligner version 3.5.2 (CodonCode Corporation) and Geneious Pro version 5.0 (Biomatters Ltd). All mtDNA sequences were unambiguously translated into their amino acid sequence (no stop codons) and no ambiguous base calling was observed. Length polymorphisms were found in all nuclear introns. Because the evolution of "indels" is difficult to model and hence incorporate into coalescent-based analysis of sequence variation, we chose to remove them from the alignments prior to further analysis. The exception was locus *15691,* for which we decided to truncate the sequence data at the first indel in the reverse direction given the presence of multiple length polymorphisms.

When the nuclear loci sequences were heterozygous at two or more nucleotide positions, we use a two-step approach to determine the gametic phase of each sequence. We first analyzed the sequences of autosomal and Z-linked loci of each individual using PHASE 2.1 (Stephens et al. 2001; Stephens and Donnelly 2003). The PHASE algorithm was run twice for each dataset (1×10^4 main iterations with a 1×10^3 burn-in, $\times100$ option) from different starting points. The gametic phase of most of the genotypes was resolved with posterior probabilities greater than 0.80 for three introns (Gapdh 95%, *15691* 100% and BRM 97% of the sequences); however, for Fib5 several individuals had allele pair probabilities <0.80. Thus, we designed allele-specific primers (3′-end matched one of the polymorphic sites in the template sequence) to amplify both alleles independently (see Bottema et al. 1993). We then employed PHASE once more, but with the additional *–k* option in effect, that is, the known gametic phases for several individuals was a priori specified, thus improving the efficiency of the algorithm: 96% ($n = 96$) of the genotypes were resolved with posterior probability >0.94.

To test for possible intralocus recombination events, we used the four-gamete test (Hudson and Kaplan 1985) as implemented in DnaSP version 5.10.1 (Librado and Rozas 2009). If recombination was detected at a given locus, all subsequent analyses were performed with the largest independently segregating block. We used DnaSP to estimate haplotype diversity (Hd, Nei 1987), nucleotide diversity (π, Nei 1987), the number of segregating sites (S, Waterson 1975), theta from S (θ, Nei 1987), and K_{ST} values (haplotype based statistics similar to F_{ST}; Hudson et al. 1992).

Microsatellites

A total of 285 birds were genotyped for 11 autosomal nuclear microsatellites specifically developed for the Karoo scrub-robin (Ribeiro et al. 2009) and multiplexed in four reactions as described in Ribeiro et al. (2011). Allele sizes of fluorescently labeled fragments were determined using the size standard LIZ-500 on an ABI 3730 DNA Analyser followed by analysis with GeneMapper version 4.0 (Applied Biosystems). Linkage disequilibrium and deviations from Hardy–Weinberg (HW) expectations, for each locus and each locality, were tested using GENEPOP version 3.4a (Raymond and Rousset 1995). Allelic diversity, Ho, He, and F_{ST} values were computed in GenAlEx version 6.1 (Peakall and Smouse 2006).

POPULATION GENETIC STRUCTURE

Two distinct approaches were used to test for population structure using the genetic data. First, we used STRUCTURE version 2.3 (Pritchard et al. 2000; Hubisz et al. 2009) to identify groups of randomly mating individuals with different microsatellite allele frequencies, by minimizing deviations from HW expectations and linkage disequilibrium. Analyses were implemented using the "admixture" model with "correlated allele frequencies,' including information about the sampling location as a prior (note that the LOCPRIOR option is different from implementing the USEPOPINFO flag; see Hubisz et al. 2009). We ran five pseudo-replicates with 1×10^6 Markov chain Monte Carlo (MCMC) iterations following a burn-in of 1×10^5. Second, to test the hypothesis that greater differentiation occurs among groups occupying distinct locals in environmental space than among groups within similar environmental niches, we used an analysis of molecular variance (AMOVA, Excoffier et al. 1992). Sequence variation (mtDNA and nuclear DNA) and allele frequency (microsatellites) were partitioned between ecologically defined groups and among populations within the previous groupings. The AMOVA was implemented in ARLEQUIN version 3.1 (Excoffier et al. 2005) and the statistical significance of the F statistics was assessed using 1×10^5 permutations.

SIGNATURES OF SELECTION IN MITHOCHONDRIAL GENES

We contend (at least for the present) that evidence for selection within species may best be inferred by implementing polymorphism-based methods. As such, sequence polymorphism data were used to test for deviations from the neutral expectation of the number of mutations (i.e., allele frequency spectrum) by estimating Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) statistics. The null distribution and statistical significance were determined by simulating the coalescence process 1×10^5 times in DnaSP.

CORRELATIVE APPROACH: COMPARING ENVIRONMENTAL, MITOCHONDRIAL GENETICS AND MORPHOLOGICAL VARIATION

Mantel matrix correspondence analysis (Mantel 1967; Smouse et al. 1986) was used to test the hypothesis that populations that occupy environmentally similar sites tend to be similar in morphology and/or in physiological genetic variation implying local

adaptation. Correlation tests were implemented as described below, using different matrices.

The effect of climate in mtDNA variation.
We estimated the correlation between an environmental variation matrix produced by estimating the linear distances along the PC1 and PC2 axes across the geographical range and a matrix representing the population molecular differentiation at mtDNA (ATPase6 and APTase8) as measured by linearized F_{ST} (F_{ST} /1–F_{ST}; Rousset 1997).

The effect of climate on morphology.
Morphological variation among populations was computed as the Euclidean distances between the residuals from the linear model fitted to tarsus-length and mass–body size. The association was tested with the climatic matrices described above.

Based on the current distribution of the species and the predicted historical ranges (Fig. 3A and supporting material: Species distribution) a historical scenario of range expansion following the Holocene Maximum (6000 years before present) was determined to be plausible. Because population densities and migration tend to decrease toward the margin of the distribution (abundant core theory; Eckert et al. 2008) the dynamics of colonization of newly available habitat from marginal populations is expected to have a strong stochastic component and thus increase differentiation. Using the relative position of each sampled locality within the species' present distribution, we expect that range expansion to have caused greater genetic differentiation among newly colonized patches than among localities sampled in the core of the species distribution.

The effect of range expansion in the mtDNA variation.
The expected differentiation based on the last dramatic change of the species range (Holocene Maximum) was coded in a trichotomous key (Fig. 3B): "0" for differentiation between populations within the stable core, "1" representing divergence between the stable core and marginal population pairs and, "2" coding the expected greater differentiation between populations in the newly colonized area. The correspondence between the observed differentiation at mtDNA genes and this hypothesis matrix was then tested.

The effect of range expansion on morphology.
Correspondence between morphological variation (as described above) and the hypothesized differentiation matrix (Fig. 3B) was tested.

All matrices were composed of the same 24 populations; nevertheless, the number of individuals used to estimate the mean population value varied according to the nature of the variable. The statistical significance of the correlation was assessed using permutations as implemented in the VEGAN package within R (Oksanen et al. 2010; R Development Core Team 2010). The distribution of the coefficient values *r,* under the null hypothesis of "no association", was obtained by shuffling the matrix values 1×10^5 times. The association was first tested between the two main matrices. We then used a partial matrix correspondence test to control for the effect of spatial proximity while still testing the possible correlation between the first two matrices. Geographical proximity between sites was measured as the Euclidean distances between log-transformed latitude/longitude coordinates, previously converted using Lambert's azimuthal equal-area projection (a projection that better represents area on a sphere, particularly important given the broad distribution of the Karoo scrub-robin).

COALESCENT ESTIMATES OF GENE FLOW

To test the role of environmental selective pressures in constraining gene flow, we used the coalescent-based "isolation-with-migration" model (Wakeley and Hey 1998; Nielsen and Wakeley 2001; Hey and Nielsen 2007) to estimate migration rates (m) between ecologically defined population pairs. The model was applied to our data (only the independently segregating segments as mentioned above) as implemented in IMa (Hey and Nielsen 2007). IMa analyses were conducted to investigate the relative gene flow across marker type, under the full six-parameter model (effective population size θ_1, θ_2, θ_3; migration m_1, m_2; time of divergence t). After preliminary runs to determine the appropriate range of priors for each of the three parameters, as well as confirming proper MCMC mixing, the final runs were performed for 2×10^7 steps with 30 MCMC-coupled chains with a geometric heating scheme (g1 = 0.3 and g2 = 0.9). The first 1×10^6 steps were discarded as the burn-in. To enable comparisons of migration rates between the three types of marker, which are characterized by different effective population sizes, we used an "inheritance scalar' to adjust the parameters in the model: (0.25—mitochondrial, 0.75—Z-linked, 1—autosomal). We then implemented the "Load-Trees" mode for the mtDNA and nDNA multilocus dataset (introns and microsatellites separately) to test the full model against a model of divergence in isolation, that is, m_1 and m_2 was restricted to zero. The significance of the test statistics (log-likelihood ratio) was assessed using a chi-squared test (Hey and Nielsen 2007). This method offers the best available option to disentangle the relative effects of lineage sorting and gene flow.

Results

ENVIRONMENTAL HETEROGENEITY

The first and second principal components accounted for 78% of the variation in the data: PC1 explained 44% and PC2 34%. The variation in PC1 is explained by differences in rainfall regimes:

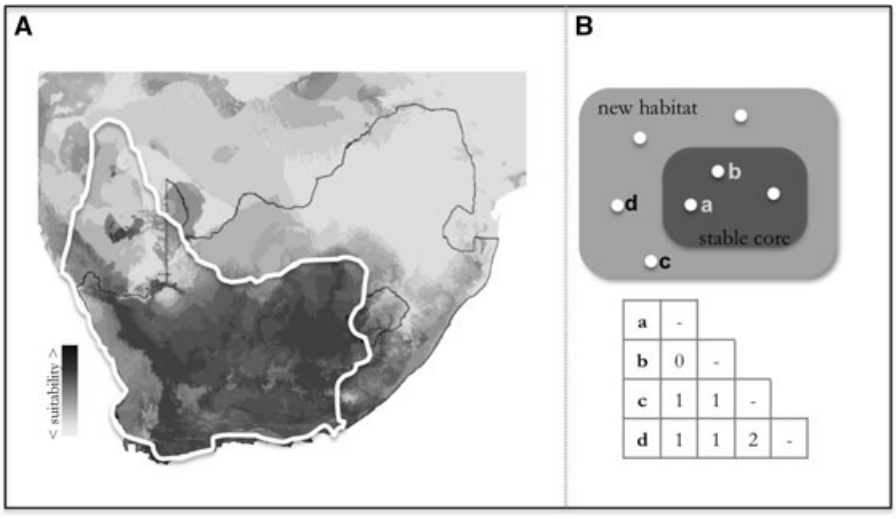

Figure 3. (A) Predicted distribution range of the Karoo scrub-robin during the Holocene. Probabilities of occurrence are depicted in a continuous scale of gray: the darker the gray the higher the likelihood of occurrence. The region outlined in white shows the current distribution of the species. (B) Colonization model and its predicted consequences for population differentiation. Dark gray represents the historical species range and light gray the current distribution. The values "0," "1," and "2" in the matrix represent the expected differentiation between populations within the stable core, divergence between the stable core and marginal population pairs, and differentiation between populations in the newly colonized area, respectively.

precipitation of the driest month (0.461) and precipitation of the driest quarter (0.452) loaded positively, whereas precipitation seasonality had a negative coefficient (–0.231). PC2 illustrates the effect of continentality: mean temperature of the coldest month (0.673) and mean temperature of the driest quarter (0.423) had the highest coefficients. A closer inspection by sampling locality revealed: (1) increasing aridity as one moves northwards, with a clear distinction between the mesic Fynbos and the xeric Karoo; (2) a sharp contrast between localities along the western coast where climate is moderated by the Atlantic Ocean, and the interior and southeast localities that exhibit a greater annual range in temperature.

MORPHOLOGICAL DIVERGENCE

A significant overall MANOVA (Wilk's $= 0.7214$, $P < 0.001$) followed by univariate analysis indicated that environmental variables had an effect on morphology. Despite the significant effect of PC1 on mass ($F_{23,270} = 7.312$, $P < 0.001$) and tarsus-length ($F_{23,270} = 7.312$, $P < 0.001$), as well as PC2 on mass ($F_{23,270} = 4.168$, $P < 0.001$) and tarsus-length ($F_{23,270} = 4.168$, $P < 0.001$), the majority of our 24 sampled populations were morphologically indistinguishable in environmental space. The few exceptions were always eco-geographically peripheral populations (Table 1). For instance birds from Grahamstown (GTN, $n = 8$) at the SE margin of the species range were heavier, whereas individuals from the Dwarskersbos population (DWK, $n = 5$) along the SW margin had the lowest body mass (see Fig. 1). A similar trend was recovered for tarsus-length with birds from Koeberg (KBG, $n = 14$) in the SW part of the range, having longer tarsi than birds from Prieska (PRK, $n = 14$) in the north central part of the species range (see Fig. 1).

PATTERNS OF GENETIC VARIATION AND SELECTION

Polymorphism, divergence estimates, frequency spectrum test of neutrality and recombination estimates for mtDNA and nDNA sequence data are summarized in Table 2. We observed substantial levels of heterogeneity in sequence variation among loci: values of π varied from 0.002 to 0.02. Genetic differentiation at the mtDNA genes among localities, K_{ST}, was very high (average 89%, $P < 0.001$). A small, but significant differentiation was also recovered for the nuclear introns: F_{ST} values varied from 2% to 14%. Within sites, all microsatellites were polymorphic, in HW and linkage equilibrium. The number of alleles ranged from three to 13; *H*o and *H*e varied from 0.581 to 0.732 and 0.658 to 0.738, respectively. Genetic differentiation ranged from 6% to 11% in our 11 microsatellites (Table S1).

The distribution of allele frequencies as measured by Tajima's D and Fu's Fs statistics varied among loci, ranging from -2.08 to 0.85 and -15.78 to -2.361, respectively. All four nuclear loci showed a significant skew in the allelic frequency spectrum, that is, a high frequency of rare polymorphisms; D and Fu's Fs were significantly different from neutral expectations (Table 2). This nucleus-wide skew toward rare alleles suggests that the Karoo scrub-robin has probably undergone a demographic expansion during the species' history. In contrast, Tajima's D for the mtDNA loci was positive and close to "1" (0.850), but

Table 1. Morphological variation across the aridity gradient as summarized using PCA: groups statistically distinguishable with post-hoc Tukey's HSD test.

	Factor	df	*F*	*P*	Groups (*n*)
Mass					
	PC1	23, 270	7.3123	<0.0001	GTN (8); DWK (5); others
	PC2	23, 270	7.3123	<0.0001	GTN (8); DWK (5); others
Tarsus-length					
	PC1	23, 270	4.1682	<0.0001	KBG (14); PRK (14); others
	PC2	23, 270	4.1682	<0.0001	KBG (14); PRK (14); others

df, *F*, and *P* from ANOVA; Three letter acronym represent sampling sites, see main text for more information; *n*: sample size.

not significant. A closer inspection of the allele frequency spectrum revealed an excess of both low frequency and high frequency polymorphism, disrupted by some intermediate frequency alleles. This is the pattern expected under positive selection: abundance of low-frequency polymorphisms and high-frequency variants (Fu 1997; Nielsen 2005).

Our most striking result is the segregation of alternate amino acids within each of the two climatically well-defined areas (as depicted in Fig. 4): two closely positioned replacement polymorphisms in the ATPase6 gene segregate in different climatic zones, whereas not a single amino acid substitution was recovered in the ATPase8 gene. The amino acid replacement I51V (Isoleucine for Valine at amino acid position 51) separated the western mesic sites from the interior arid populations, hereafter referred to as Western and Central groups. Isoleucine (I) was almost exclusively present in populations that experience relatively small variations in mean annual temperature due to the moderating influence of the nearby Atlantic Ocean and receive most of their rainfall during the cold season. In contrast, Valine (V) at position 51 was recovered for all individuals sampled from the portion of the species range characterized by a summer rainfall regime (see also Fig. 1 from Chase and Meadows 2007). One site was exceptional: Bontebok (BTB, n = 4) where Valine was dominant (100%). Although not analyzed in this article given the small sample size ($n = 2$), it is worth mentioning that Helderberg was the single site where we found the co-occurrence of both forms of ATPase6 molecules (50% V, 50% I; depicted in Fig. 4). An additional amino acid change at H63D was recovered exclusively within the most mesic area within the winter rainfall domain. Populations in this section of the environmental space have an Aspartic Acid (D) in position 63, as opposed to the remaining populations, which have a Histidine (H): the two forms of this replacement of ATPase6 are only sympatric at Beaverlac (62% D, 38% H, $n = 8$; Fig. 4).

CONTEMPORARY GENETIC STRUCTURE AND GENE FLOW

Overall, F_{ST} values estimated for our microsatellite data were small (<0.088) with the two most distant populations (aerial distance ≈ 1200km) having a $F_{ST} = 0.056$. No signal of isolation by distance ($r = 0.103$, $P = 0.283$) was recovered. These results were further supported by the lack of genetic clustering found with the Bayesian clustering method implemented in STRUCTURE, which indicated high levels of gene flow between populations: the likelihood of the marginal posterior probability distribution

Table 2. Summary of the genetic variation in sequence data: mtDNA, autosomal loci, and the Z-linked locus

Genome		Loci (*n*)	Sites (bp)	*R*	S	H	Hd	π	$\theta^{\#}$	*D*	Fu's Fs	K_{ST}
mtDNA		ATP8 (164)	168	–	8	9	0.627	0.009	0.008	0.1205	−0.728	
		ATP6 (164)	578	–	61	39	0.856	0.025	0.019	0.9212	−0.732	
		All	754	–	70	46	0.870	0.021	0.017	0.8497 ($P = 0.865$)	−2.361 ($P = 0.370$)	0.89**
nDNA	Autosomal	GAPDH (214)	194	0	10	10	0.152	0.001	0.009	−2.0854 ($P < 0.001$)	−15.7830 ($P = 0.001$)	
			262	1	14	15	0.692	0.004	0.090	−1.5126 ($P = 0.031$)	−9.205 ($P = 0.015$)	0.05*
		FIB5 (184)	307	0	15	15	0.613	0.003	0.008	−1.6842 ($P = 0.01$)	−9.9294 ($P = 0.005$)	
			465	2	30	35	0.899	0.087	0.011	−0.6040 ($P = 0.329$)	−13.6781 ($P = 0.005$)	0.05*
		15691 (180)	135	0	8	8	0.235	0.002	0.010	−1.76876 ($P = 0.001$)	−7.272 ($P = 0.019$)	0.02
	Z -linked	BRM (193)	257	0	11	13	0.511	0.002	0.008	−1.7243 ($P = 0.003$)	−10.352 ($P < 0.001$)	0.14**

***n* = number of alleles; bp = base pairs; R = number recombination events, S = segregating sites; H = number of haplotypes; Hd = haplotype, and π = nucleotide diversity, $\theta^{\#}$ = theta per site, *D* = Tajima's D; and K_{ST} = genetic divergence (Hudson et al. 1992).**
*** $P < 0.01$, ** $P < 0.001$.**

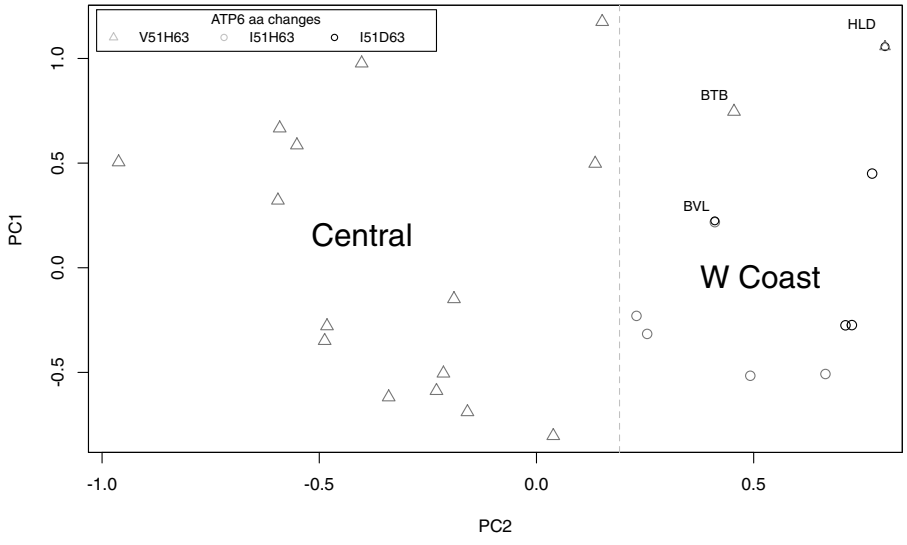

Figure 4. Environmental space of the two amino acid replacements, Isoleucine/Valine at amino acid 51 and Histidine/Aspartic Acid at amino acid 63, in the OXPHOS genes. Sites mention in the main text: Bontebock (BTB), Helderberg (HLD), and Beaverlac (BVL).

was the highest when $K = 1$ (Figure S1). The low F_{ST} values observed hinder the implementation of BayesAss to accurately estimate current gene flow.

ECOLOGICAL DIVERGENCE: CORRELATION BETWEEN PHENOTYPE, GENOTYPE AND ENVIRONMENT

The AMOVA results confirmed that most of the mitochondrial variation was associated with environmental PC2-based groupings: 77% ($F_{CT} = 0.77$, $P < 0.001$) between the two abovementioned groups (Western vs. Central; see Fig. 4) when compared with 1.1% among localities within each group ($F_{SC} = 0.658$, $p < 0.001$). It also revealed, nonetheless, that a significant but small portion of the variation was explained by differences among all localities (7.77%; $F_{ST} = 0.922$, $P < 0.001$). In contrast, our intron data indicated that variance among groups was low and only significant at one locus: 0% for BRM ($P = 0.954$), 0.7% for Fib5 ($P = 0.345$), 0.1% for Gapdh ($P = 0.157$) and 4.6% for *15691* ($P = 0.015$). Most of the variation was rather significantly partitioned among all localities: BRM = 83% ($F_{ST} = 0.170$, $P < 0.001$), Fib5 = 95.6% ($F_{ST} = 0.04$, $P = 0.020$), Gapdh = 93.6%, ($F_{ST} = 0.06$, $P = 0.010$) and *15691* = 97.4% ($F_{ST} = 0.015$, $P < 0.015$). The analysis of variance of microsatellites alleles revealed a similar result, that is, the hypothesis that population structure was associated with climate groupings was not supported (0.02%, $F_{CT} = 0.002$, $P = 0.38$) and most of the variation, 90.3%, was distributed among all localities ($F_{ST} = 0.039$, $P < 0.001$). Note that the AMOVA has an inherent spatial effect, which might partially affect the results. To remove the spatial proximity effect, we used a Mantel test of matrix correspondence. A significant portion of mtDNA and morphological divergence could be explained by the spatial distribution of the environmental conditions (37% and 34%, respectively). However, as expected, spatially close sites were characterized by similar environmental conditions ($r = 0.607$ $P < 0.001$). Once we removed the effect of spatial proximity, PC2 still explained 39% ($P = 0.008$) of the mtDNA haplotypic distribution. In contrast, morphology was associated with geographical distance: populations far apart were phenotypically more distinct. This is consistent with the idea that the effect of migration decreases from the core toward the edge of the distribution where drift plays a greater role (Table 3). Genetically (mtDNA genes) divergent populations were not generally morphologically divergent from each other ($r = -0.077$, $P = 0.752$). There was a significant correspondence (27.8%, $P = 0.011$) between observed mtDNA variation and the hypothesis matrix (Fig. 3) indicating that genetic variation among newly colonized populations is higher than between populations from the stable core. Thus, suggesting the possible role of drift during colonization, in creating the observed pattern in mtDNA. No correlation was, however, found between the hypothesis matrix and morphological divergence ($r = -0.068$, $P = 0.648$). Overall, and after Bonferroni corrections, only mtDNA genetic variation correlated with environmental and range dynamics.

THE EFFECT OF ENVIRONMENTAL HETEROGENEITY ON GENE FLOW

We found opposite trends in gene flow according to the inheritance mode of the genetic marker (Fig. 5). Migration rate (m) from Western to Central populations, for mtDNA, was 0.075 (0.015–0.2550, 90% high posterior density (HPD) interval; Fig. 5C). In the opposite direction, the migration rate was 0.005 (90% HPD: 0.005–0.115, note that 0.005 was the first bin of the prior and therefore corresponds to zero; Fig. 5D). Estimates were higher

Table 3. **Coefficients from matrix correlation tests depicting the degree of congruence between environmental variation and mitochondrial divergence, as well as morphological patterns. Significant values are in bold.**

	Space	Environment		
	GGD	PC1	PC2	Historical range shift
Physiological genetics	0.100 ($P = 0.102$)	−0.214 ($P = 0.998$)	**0.396** ($P < 0.001$)	**0.278** ($P = 0.011$)
| GGD	−0.288 ($P = 1.000$)	**0.388** ($P = 0.008$)*	**0.278** ($P = 0.011$)*
Morphology	**0.295** ($P = 0.002$)	**0.263** ($P = 0.01$)	**0.278** ($P = 0.004$)	−0.097 ($P = 0.711$)
| GGD	0.154 ($P = 0.071$)	**0.192** ($P = 0.038$)	−0.068 ($P = 0.648$)

" | " is used to delineate that the given variable was controlled in a partial matrix correlation test. GGD stands for geographical distance.
***Significant results after Bonferroni correction for multiple tests ($\alpha = 0.05/3 = 0.017$).**

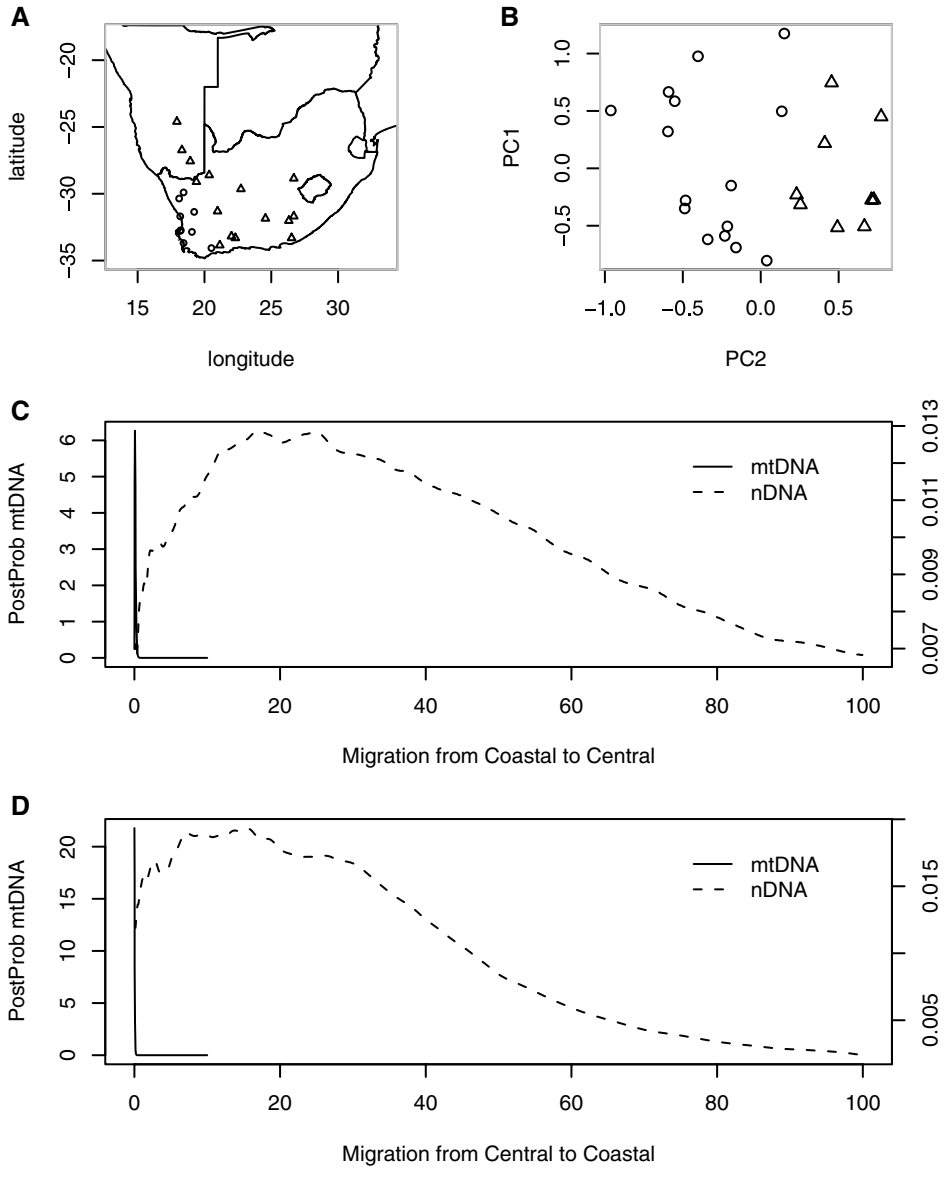

Figure 5. **Coalescent estimates of migration for the two environmentally defined groups: geographical (A) and environmental (B) space for each group. (C) Eastwards migration m_1 (from Western Coast to Central) and (D) Westwards migration m_2 (from Central to Western Coast).**

Table 4. Likelihood ratio tests for a series of nested models applied to the ecologically defined groups (see Fig. 5A,B). Divergence in isolation or directional migration was tested against the full (six-parameter) model

Migration	Model	Data	logL	df	2LLR
Isolation	$\theta_1 = \theta_2 = \theta_a; m_1 = m_2 = 0$	mtDNA	−13.569	4	17.8851*
		nDNA	−460.517	4	916.669*
	$\theta_1, \theta_2, \theta_a; m_1 = m_2 = 0$	mtDNA	−12.503	2	15.2833*
		nDNA	−460.517	2	916.669*
	$\theta_1 = \theta_2, \theta_a; m_1 = m_2 = 0$	mtDNA	−13.306	3	16.5423*
		nDNA	−460.517	3	916.669*
	$\theta_1, \theta_2 = \theta_a; m_1 = m_2 = 0$	mtDNA	−13.693	3	17.3574*
		nDNA	−460.517	3	916.669*
	$\theta_2, \theta_1 = \theta_a; m_1 = m_2 = 0$	mtDNA	−13.693	3	17.3574*
		nDNA	−460.517	3	916.669*
Directional	$\theta_1, \theta_2, \theta_a; m_1, m_2 = 0$	mtDNA	−5.0139	1	−0.0002
		nDNA	−4.042	1	3.720
	$\theta_1, \theta_2, \theta_a; m_1 = 0, m_2$	mtDNA	−12.656	1	15.2838*
		nDNA	−7.439	1	10.515*

*significant after Bonferroni correction for multiple comparisons ($\alpha_{new} = 0.0065$).

for the nuclear introns: from Western to Central $m = 17.05$ (90% HPD: 0.55–86.25) and from Central to Western $m = 15.45$ (90% HPD: 0.05 – 68.95). These estimates were 50 times higher than the values obtained for mtDNA genes, even after rescaling according to the effective population size (fourfold Ne). For microsatellites, m estimates were even higher (>100) than the values obtained with the intron data. The LLR tests clearly rejected a model of divergence in isolation ($m_1 = m_2 = 0$) whether analyzing the four nuclear introns, the 11 microsatellites, or the mtDNA data (Table 4). It is worth noting that the models that set gene flow from the inland Central to the Western group to zero ($m_2 = 0$) could not be rejected for the mtDNA genes and intron data (Table 4). Together with the microsatellite data, this asymmetrical gene flow indicates that the movement of alleles inland occurs at a greater frequency than the movement of alleles from inland to Western populations. These results suggest that mitochondrial divergence is most likely due to low levels of maternal gene flow (despite possible extensive migration of females) than to past population demography, which due to the more rapid coalescence of mtDNA may have resulted in faster sorting of alleles.

Discussion

Ecological transitions have long been recognized as important for examining the influence of differential selection in space (e.g., Levene 1953; Endler 1986). Yet, empirical contributions (e.g., Smith et al. 1997/2001; Rosenblum 2006; Milá et al. 2009) are lagging, partially due to the way neutral theory (Kimura 1983) has inadvertently shaped the mind-set of population genetic empiricists over the past three decades. In this study, we have combined different lines of evidence (as illustrated in Fig. 6) to demonstrate that the range of the Karoo scrub-robin, as a whole, comprises several local evolutionary domains: (1) the potential for local selection to occur due to geographically varying climatic conditions, (2) sharp eco-geographical transition of mitochondrial variants, which occupy a vast geographical area, (3) nonrandom distribution of amino acids with differing physicochemical proprieties (solubility and polarity) in distinct eco-geographically defined areas, (4) reduced effective migration at mtDNA among ecologically divergent groups, (5) extensive gene flow as quantified with neutral genetic markers and, (6) lack of morphological divergence. The genetic variation observed in the mtDNA likely result from differential metabolic rates among individuals occupying different portions of the range, that is, local adaptation.

LOCAL ADAPTATION: MIGRATION–SELECTION BALANCE

The results presented here are consistent with the migration–selection model: strong natural selection maintaining alternative mtDNA haplotypes in populations occupying different ecological conditions, despite gene flow at neutral loci. The coalescent models of divergence with gene flow revealed a significantly better fit to the data compared to models with no gene flow (Table 4). The 90% high posterior density interval of migration as estimated with the mtDNA and intron data included "zero" from the Central to the Western group. Further, the analysis of nested models did not allow us to reject the model of asymmetrical gene flow. The possible absence of gene flow westwards might reflect differences in fitness of individuals in mismatched environments. Remarkably different estimates of gene flow between nonadaptive (nuclear)

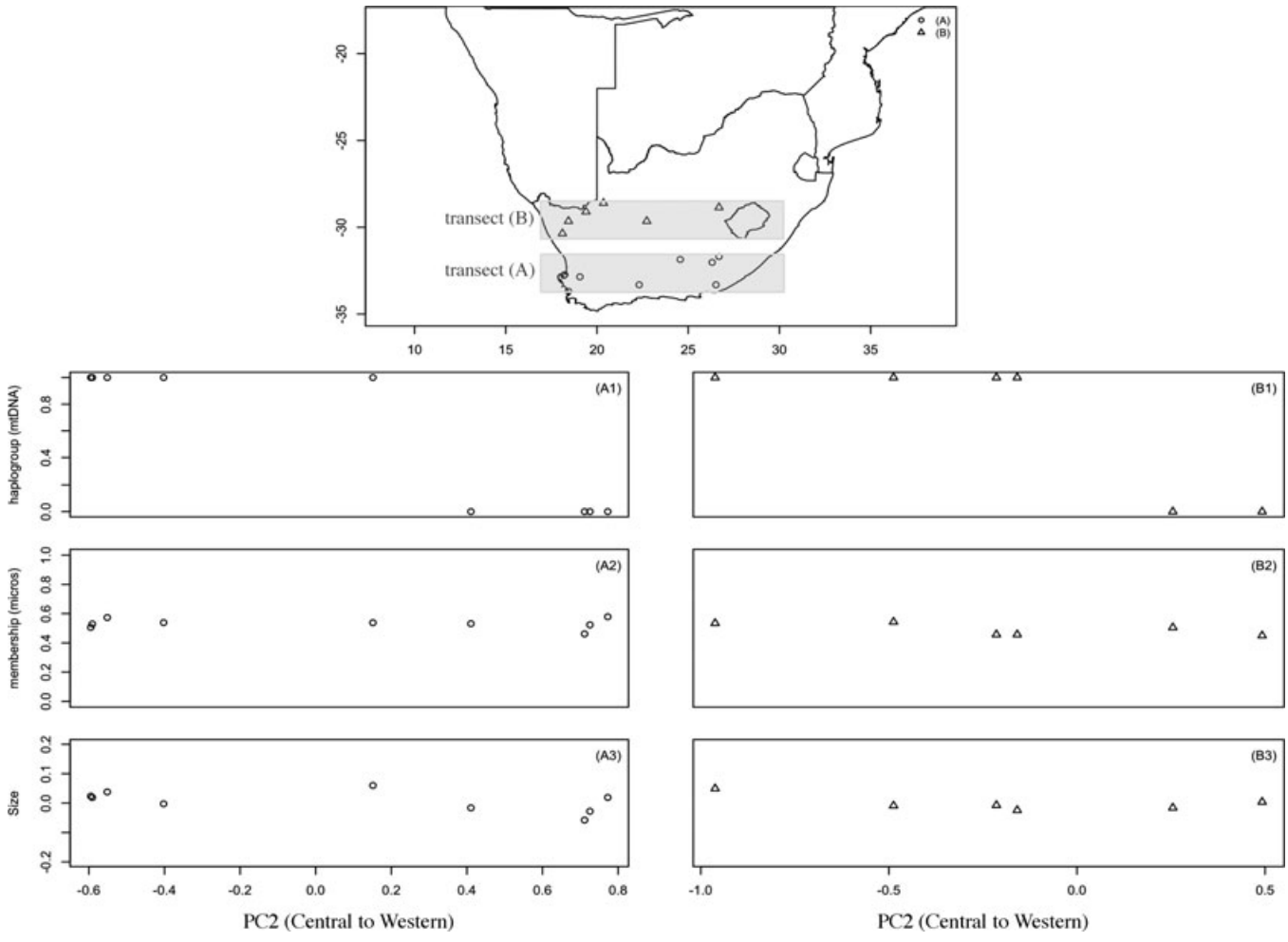

Figure 6. Physiological (mtDNA haplogroup membership), neutral genetic (population membership as estimated for microsatellites using STRUCTURE), and morphological variation (size measured as regression of mass on tarsus) along two independent Central–Western independent transects (A) and (B).

and adaptive (mtDNA) genes support our hypothesis. When scaled for the effective population size, mitochondrial gene flow was at least 50-fold smaller than expected under a migration–drift model (e.g., from Western to Central group: nDNA $m = 4.26$, whereas mtDNA $m = 0.075$).

Although our intron data likely violate the assumptions of population demographic stability (negative Tajima's D) of the "isolation-with-migration" model, and thus the absolute estimate of nonadaptive gene flow may not be accurate, it was recently demonstrated that this model is quite robust to such violation (Strasburg and Rieseberg 2010). Furthermore, under a scenario of population growth, parameter estimates other than population size were comparable to the stable demographic scenario (Strasburg and Rieseberg 2010). Because we were primarily interested in gene flow estimates, we consider this violation very unlikely to significantly bias our results. The fact that only four eco-geographical peripheral populations were significantly different in morphological traits and hence that sites from throughout most of the species distribution were similar in body mass and tarsus-length lends further support to the homogenizing effect of recurrent gene flow in shaping the current patterns of variation among nonadaptive traits.

The hypothesis that changes in mtDNA OXPHOS genes might be a common mechanism for facilitating rapid adaptation to particular environmental conditions has received increasing support from intraspecific studies (birds: Cheviron and Brumfield 2009; humans: Balloux et al. 2000). However, identifying the actual gene(s) underlying any adaptive response is a challenging task. Changes in the physicochemical features of one or a few amino acids can affect the function/structure of a given enzyme/protein (e.g., MC1R: Theron et al. 2001; Violet-sensitive Opsin: Shi and Yokoyama 2003; *Agouti*: Linnen et al. 2009) and thus have dramatic implications in the physiological pathway in which they are involved. The population genetics approach implemented here (i.e., allele frequency spectrum) revealed the increase of mutations at high frequencies, which might be a signal of

positive selection or alternatively, a possible mimicking effect of past demographic dynamics.

DIVERGENCE: SELECTION VERSUS DEMOGRAPHY

The observed discrepancy between the nuclear and mitochondrial genomes is consistent with the expectations of a model where selection is strong and acts on a single trait (Nosil et al. 2009). An appealing argument with which to explain the maintenance of such strong spatial structure in the mtDNA haplotypes, in the absence of a current geographical barrier to gene flow, would be male-biased dispersal. However, this is unlikely for this species given the results from direct and indirect measures of gene flow, which have demonstrated that in the Karoo scrub-robin, females (as in birds generally; Greenwood 1980) are the long-distance dispersers, whereas male dispersal distance follows a leptokurtic distribution (Ribeiro et al. 2011).

The type of genetic clines observed here (see Fig. 6A1, B1) can also be formed by the diffusion of alleles after a period of divergence in isolation (Endler 1977). However, this seems an unlikely explanation, for the following reasons. First, the geological history of the area offers no plausible scenario for the model of divergence-in-isolation because the most recent geological event, establishment of the present-day shape of the Great Escarpment dates to the late Miocene (Partridge 1997). Second, the hypothesis of allopatric divergence due to habitat unsuitability via climatic cycling during the Plio–Pleistocene (e.g., Matthee and Flemming 2002; Herron et al. 2005; Tolley et al. 2006; Outlaw et al. 2007; Swart et al. 2009) was not supported by our historical geographical range models, which only predicted a single refugium.

Such a sharp change in allele frequencies can also be a consequence of a range expansion, a phenomenon known as "surfing" (Excoffier and Ray 2008). The comparison between the predicted historical and current ranges suggests a range expansion westward, after the Holocene maximum. During the expansion, rare mtDNA variants "surfing" on the front of the colonization "wave" might have increased in frequency due to strong genetic drift. The genetic signature observed in the nuclear genome (i.e., significant negative Tajima's *D* and Fu's Fs) as well as the location of morphologically different populations at the trailing (GTN and PRK) and leading (KBG and DWK) edges of the moving range, gives further support to the hypothesis of a range expansion out of a single refugium. Nonetheless, secondary contact or "surfing" of alleles during expansion fails to explain the maintenance of the spatial segregation of mitochondrial haplotypes despite extensive gene flow (introns and microsatellites). In the absence of any spatial difference in the survival of genotypes or asymmetrical competition for some kind of resource (Goldberg and Lande 2007), theory predicts that two groups will merge into each other's range as a function of time since contact *t,* scaled by dispersal distances per generation σ (neutral diffusion model; Endler 1977). Therefore, the spatial difference in gene frequencies will disappear as a consequence of the swamping effect of gene flow. In this particular system where females are the long-distance disperser and the mean velocity of the spread of maternal alleles is quite high (σ females = 892 m/gen; Ribeiro et al. 2011) the merger between the previously diverged populations should be well underway. Given all of the above, different evolutionary forces may be responsible for creating and maintaining the observed variation: mutation creating new haplotypes, drift perhaps helping to increase their frequency in the population, and selection "trapping" the haplotypes that are locally adapted.

EXTREME ENVIRONMENTS

The southern African arid and semi-arid biomes have a unique combination of environmental parameters, particularly highly seasonal rainfall, which affects resource availability, such as water and food. Nevertheless, and despite their high mass-specific metabolism and high body temperature, some passerine birds are permanent residents in such harsh environments. Empirical data have demonstrated that selection favors reduction of the amount of daily energy required to sustain vital metabolic pathways in birds living in such areas (Tieleman et al. 2003) and also that the mitochondrial genome is partially involved in the control of the metabolic rate in birds (Tieleman et al. 2009). In this context, our results suggest that populations might have adapted physiologically, with minimal morphological change. EWL in the Karoo scrub-robin can be reduced by mechanisms other than surface area reduction. For instance, by reducing the BMR birds are also reducing water loss (Williams and Tieleman 2005). The Western group is distributed over an area where food is available almost all-year round with its abundance peaking during winter. In contrast, in the central area, food and water are scarce during the winter (when temperatures can be below freezing). This poses an extra challenge to the interior birds. Any mitochondrial variant that would allow individuals to meet the daily energetic requirements to regulate body temperature and reduce water loss would be advantageous in such extremely cold and less-productive areas. Because the mitochondrial genome is transmitted as a unique linkage block, the amino acid replacements observed in the F0 domain of the ATP synthase can have an adaptive function or alternatively be hitchhiking with variation in other OXPHOS gene(s) upon which selective pressures might be acting.

Together with physiology, behavioral plasticity in seasonal and daily selection of microhabitats that provide protection from the heat and cold might also play an important role in allowing birds to survive the drastic temperature amplitude and potential starvation in these harsh arid environments. Further investigation that would allow us to put the current findings in the context of the causal effects on individual fitness seems promising, especially if we are to understand the genetic, physiological, and behavioral

basis of adaptation to xeric environments. Understanding adaptation to aridity has become of critical importance as desertification and drought are expected to increase and hence affect the distribution of many species worldwide, and in particularly the terrestrial animal species of western and central southern Africa (Erasmus et al. 2002).

ACKNOWLEDGMENTS

We thank J. Fuchs, G. Oatley, H. Smit, and R. Lopes for their assistance in the field. Thoughtful comments from A. Carnaval, A. Rush, J. McEntee, J. Fong, M. Fujita, R. Damasceno, two anonymous reviewers, and the associated editor A. Kopp greatly improved the manuscript. Other members of the Bowie lab are also thanked for discussion. Research and collecting permits were granted by the following agencies: South African National Parks, South African Provincial Departments, and the Department of Environment and Tourism of Namibia. The capture and sampling was conducted with approval of the Animal Use committees of the University of California at Berkeley, the University of Cape Town and SAFRING. For providing samples, we are grateful to David Willard, Gary Voelker and Tom Gnoske (Field Museum, Chicago, USA), and Dawie de Swart (National Museum at Bloemfontein, Free State, South Africa). The IMa analyses were partially performed using the Computational Biology Service Unit at Cornell University. We acknowledge the *Fundação para a Ciência e Tecnologia* for the Ph.D. fellowship (SFRH/BD/27739/2006) awarded to Ângela M. Ribeiro. NSF (DEB 0613668) and a Centre of Excellence grant from the Department of Science and Technology and National Research Foundation (South Africa) collectively funded some of the fieldwork.

LITERATURE CITED

Backström, N., S. Fagerberg, and H. Ellegren. 2008. Genomics of natural bird populations: a gene-based set of reference markers evenly spread across the avian genome. Mol. Ecol. 17:964–980.

Ballard, J., and M. C. Whitlock. 2004. The incomplete natural history of mitochondria. Mol. Ecol. 13:729–744.

Ballard, J., and R. Melvin. 2010. Linking mitochondrial genotype with organismal phenotype. Mol. Ecol. 119:1523–1539.

Balloux, F., L. J. L. Handley, T. Jombart, H. Liu, and A. Manica. 2009. Climate shaped the worldwide distribution of human mitochondrial DNA sequence variation. Proc. R. Soc. Lond. B 276:3447–3455.

Bottema, C. D. K., G. Sarkar, J. D. Cassady, S. Ii, C. M. Duttonand, and S. S. Sommer. 1993. Polymerase chain-reaction amplification of specific alleles: a general method of detection of mutations, polymorphisms, and haplotypes. Meth. Enzymol. 218:388–402.

Bulmer, M. G. 1972. Multiple niche polymorphism. Am. Nat. 106:254–257.

Chase, B. M., and M. E. Meadows. 2007. Late Quaternary dynamics of southern Africa's winter rainfall zone. Earth Science Rev. 84:103–138.

Cheviron, Z. A., and R. T. Brumfield. 2009. Migration-selection balance and local adaptation of mitochondrial haplotypes in Rufous-collared Sparrows (*Zonotrichia capensis*) along an elevational gradient. Evolution 63:1593–1605.

Dean, W. R. J., and Milton, S. J. (Eds.), 2004. The Karoo: ecological patterns and processes. Cambridge Univ. Press, Cambridge.

Desmet, P. G., and Cowling R. M. 1999. Biodiversity, habitat and range-size aspects of a flora from a winter-rainfall desert in north-western Namaqualand, South Africa. Plant Ecol. 142:23–33.

Doebeli, M., and U. Dieckmann. 2003. Specation along environmental gradients. Nature 421:254–264.

Eckert, C. G., K. E. Samis, and S. C. Lougheed. 2008. Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. Mol. Ecol. 17:1170–88.

Endler, J. 1977. Geographic variation, speciation and clines. Princeton Univ. Press, Princeton, NJ.

———. 1986. Natural selection in the wild. Princeton Univ. Press, Princeton, NJ.

Erasmus, B. F. N., A. S. Van Jaarsveld, S. L. Chown, M. Kshatriya, and K. J. Wessels. 2002. Vulnerability of South African animal taxa to climate change. Global Change Biol 8:679–693.

Excoffier, L., and N. Ray. 2008. Surfing during population expansions promotes genetic revolutions and structuration. Trends Ecol. Evol. 23:347–351.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.1: an integrated software package for population genetics data analysis. Evol. Bioinformatics Online 1:47–50.

Excoffier, L., P. Smouse, and J. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Fridolfsson, A. K., and H. Ellegren. 1999. A simple and universal method for molecular sexing of non-ratite birds. J. Avian Biol. 30:116–121.

Friesen, V. L., B. C. Congdon, H. E. Walsh, and T. P. Birt. 1997. Intron variation in Marbled Murrelets detected using analyses of single-stranded conformation polymorphisms. Mol. Ecol. 6:1047–1058.

Fu, Y. X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925.

Fuchs, J., R. C. K. Bowie, J. Fjeldsa, and E. Pasquet. 2004. Phylogenetic relationships of the African Bush-shrikes and Helmet-shrikes (Passeriformes: Malaconotidae). Mol. Phylogenet. Evol. 33:428–439.

Grinnell, J. 1924. Geography and Evolution. Ecology 5:225–229.

Gershoni, M., A. R. Templeton, and D. Mishmar. 2009. Mitochondrial bioenergetics as a major motive force of speciation. BioEssays 31:642–650.

Goldberg, E. E., and R. Lande. 2007. Species' borders and dispersal barriers. Am. Nat. 170:297–304.

Goodwin, G. H. 1997. Isolation of cDNAs encoding chicken homologues of the yeast SNF2 and Drosophila Brahma proteins. Gene 184:27–32.

Greenwood, P. J. 1980. Mating systems, philopatry and dispersal in birds and mammals. Anim. Behav 28:1140–1162.

Herron, M. D., J. M. Waterman, and C. L. Parkinson. 2005. Phylogeny and historical biogeography of African ground squirrels: the role of climate change in the evolution of *Xerus*. Mol. Ecol. 14:2773–2788.

Hey, J., and R. Nielsen. 2007. Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. Proc. Natl. Acad. Sci. USA 104:2785–2790.

Hijmans, R. J., J. Guarino, C. Bussink, P. Mathur, M. Cruz, I. Barrentes, and E. Rojas. 2004. DIVA-GIS ver. 4.0. A geographic information system for the analysis of species distribution data. Bioinformatics 19:2496–2497.

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25:1965–1978.

Hochachka, P. W., and G. N. Somero. 2002. Biochemical adaptation: mechanism and process in physiological evolution. Oxford Univ. Press, Oxford.

Hubisz, M. J., D. Falush, M. Stephens, and J. K. Pritchard. 2009. Inferring weak population structure with the assistance of sample group information. Mol. Ecol. Res. 9:1322–1332.

Hudson, R. R., and N. L. Kaplan. 1985. Statistical properties of the number of recombination events in the history of a sample of DNA sequences. Genetics 111:147–164.

Hudson, R. R., M. Slatkin, and W. P. Maddison. 1992. Estimation of levels of gene flow from DNA sequence data. Genetics 132:583–589.

Kimball, R. T., E. L. Braun, F. K. Barker, R. C. K. Bowie, M. J. Braun, J. L. Chojnowski, S. J. Hackett, K.-L. Han, J. Harshman, V. Heimer-Torres, et al. 2009. A well-tested set of primers to amplify regions spread across the avian genome. Mol. Phylogenet. Evol. 50:654–660.

Kimura, M. 1983. The neutral theory of molecular evolution. Cambridge Univ. Press, Cambridge, UK.

Lenormand, T. 2002. Gene flow and the limits to natural selection. Trends Ecol. Evol. 117:183–189.

Levene, H. 1953. Genetic equilibrium when more than one ecological niche is available. Am. Nat. 87:331–333.

Librado, P., and J. Rozas. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452.

Lillywhite, H. B., and C. A. Navas. 2006. Animals, energy and water in extreme environments: Perspectives from Ithala 2004. Physiol. Biochem. Zool. 79:265–273.

Linnen, C. R., E. P. Kingsley, J. D. Jensen, and H. E. Hoekstra. 2009. On the origin and spread of an adaptive allele in deer mice. Science 325:1095–1098.

Mantel, N. A. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27:209–220.

Matthee, C. A., and A. F. Flemming. 2002. Population fragmentation in the southern rock agama, *Agama atra*: more evidence for vicariance in southern Africa. Mol. Ecol. 11:465–471.

McCracken, K. G., M. Bulgarella, K. P. Johnson, M. K. Kuhner, J. Trucco, T. H. Valqui, R. E. Wilson, and J. L. Peters. 2009. Gene flow in the face of countervailing selection: adaptation to high altitude hypoxia in the βA hemoglobin subunit of yellow-billed pintails in the Andes. Mol. Biol. Evol. 26:815–827.

Milá, B., R. K. Wayne, P. Fitze, and T. B. Smith. 2009. Divergence with gene flow and fine-scale phylogeographic structure in the wedge-billed woodcreeper *Glyphorynchus spirurus*, a Neotropical rainforest bird. Mol. Ecol. 18:2879–2995.

Mishmar, D., E. Ruiz-Pesini, P. Golik, V. Macaulay, A. G. Clark, S. Hoseini, M. Brandon, K. Easely, E. Chen, M. D. Brown, et al. 2003. Natural selection shaped regional mtDNA variation in humans. Proc. Natl. Acad. Sci. USA 100:171–176.

Mullen, L. M., and H. E. Hoekstra. 2008. Natural selection along an environmental gradient: a classic cline in mouse pigmentation. Evolution 62:1555–1569.

Nachman, M. 2006. Detecting selection at the molecular level. Pp. 103–118 *in* C. W. Fox and J. B. Wolf, eds. Evolutionary genetics, concepts and case studies. Oxford Univ. Press, Oxford.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York, NY.

Nielsen, R. 2005. Molecular signatures of natural selection. Annu. Rev. Genet. 39:197–218.

Nielsen, R., and J. Wakeley. 2001. Distinguishing migration from isolation: a Markov chain Monte Carlo approach. Genetics 158:885–896.

Nix, H. A. 1986. A biogeographical analysis of Australian elapid snakes. Pp. 4–10 *in* R. Longmore, ed. Snakes: atlas of elapid snakes of Australia. Australian Bureau of Flora and Fauna. Canberra.

Nosil, P., D. J. Funk and D. Ortiz-Barrientos. 2009. Divergent selection and heterogeneous genomic divergence. Mol. Ecol. 18:375–402.

Oatley, T., and G. Arnott. 1988. Robins of Africa. Acorn books and Russel Friedman books. Halfway House. South Africa.

Ogden, R., and R. S. Thorpe. 2002. Molecular evidence for ecological speciation in tropical habitats. Proc. Natl. Acad. Sci, USA 99:13612–13615.

Oksanen, J., R. Kindt, P. Legendre, B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner, 2008. VEGAN: community ecology package. R package version 1:15–1.

Outlaw, R. K., G. Voelker, and D. C. Outlaw, 2007. Molecular systematics and historical biogeography of the rock-thrushes (Muscicapidae: *Monticola*). Auk 124:561–577.

Partridge, T. C. 1997. Evolution of landscapes. Pp. 5–20 *in* R. M. Cowling, D. M. Richardson and S. M. Pierce, eds. Vegetation of Southern Africa. Cambridge Univ. Press, Cambridge.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for research and teaching. Mol Ecol Notes 6:288–295.

Pedersen, P. L., Y. H. Ko, and S. Hong. 2000. ATP Synthesis in the year 200: Defining the different levels of mechanism and getting a grip on each, J Bioenerg. Biomemb. 32: 423–432.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 3.4): population genetics software for exact tests and ecumenicism. J. Heredity 86:248–249.

Ribeiro, Â. M., P. Lloyd, K. A. Feldheim, and R. C. K. Bowie. 2009. Microsatellites in the Karoo Scrub-robin, *Cercotrichas coryphaeus* (Passeriformes: Muscicapinae): isolation and characterization of 13 autosomal and two sex-linked loci. Mol. Ecol. Res. 9:636–638.

Ribeiro, Â. M, P. Lloyd, K. A. Feldheim, and R. C. K. Bowie. 2011. Microgeographical socio-genetic structure of an African cooperative breeding passerine revealed: integrating behavioural and genetic data. Mol. Ecol. accepted.

Ricklefs, R. E., and M. Wikelski. 2002. The physiology/life history nexus. Trends Ecol. Evol. 17:462–468.

Rosenblum, E. B. 2006. Convergent evolution and divergent selection: lizards at the white sands ecotone. Am. Nat. 167:1–15.

Rosenblum, E. B., M. Hickerson, and C. Moritz. 2007. A multilocus perspective on colonization accompanied by selection and gene flow. Evolution 61:2971–2985.

Rousset, F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145:1219–1228.

Scott, G. R., P. M. Schulte, S. Egginton, A.L.M. Scott, J. G. Richards, and W. K. Milsom 2011. Molecular evolution of cytochrome C oxidase underlies high-altitude adaptation in the bar-headed goose. Mol Biol Evol. 28:351–363.

Smith, T. B., R. K. Wayne, D. J. Girman, and M. W. Bruford. 1997. A role for ecotones in generating rainforest biodiversity. Science 276:1855–1857.

Smith, T. B., C. J. Schneider, and K. Holder. 2001. Refugial isolation versus ecological gradients: testing alternative mechanisms of evolutionary divergence in four rainforest vertebrates. Genetica 112:383–398.

Smouse, P. E., J. Long, and R. R. Sokal. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Syst. Zool. 35:627–632.

Shi, Y., and S. Yokoyama. 2003. Molecular analysis of the evolutionary significance of ultraviolet vision in vertebrates. Proc. Natl. Acad. Sci. USA 100:8308–8313.

Stephens, M., and P. Donnelly. 2003. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. Am. J. Hum. Genet. 73:1162–1169.

Stephens, M., N. Smith, and P. Donnelly. 2001. A new statistical method for haplotype reconstruction from population data. Am. J. Hum. Genet. 68:978–989.

Storz, J. F., S. J. Sabatino, F. G. Hoffmann, E. J. Gering, H. Moriyama, N. Ferrand, B. Monteiro, and M. W. Nachman. 2007. The molecular basis of high-altitude adaptation in deer mice. Plos Genet. 3:448–459.

Strasburg, J. L., L. H. Rieseberg. 2010. How robust are "Isolation with Migration" analyses to violations of the IM model? A simulation study. Mol. Biol. Evol. 27:297–310.

Swart, B. L., K. A. Tolley, and C. A. Matthee. 2009. Climate change drives speciation in the southern rock agama (*Agama atra*) in the Cape Floristic Region, South Africa. J. Biogeogr. 36:78–87.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

Tieleman, B. I., J. B. Williams, and P. Bloomer. 2003. Adaptation of metabolism and evaporative water loss along an aridity gradient. Proc. R. Soc. Lond. B 270:207–214.

Tieleman B. I., M. A. Versteegh, A. Fries, B. Helm, N. J. Dingemanse, H. L. Gibbs, and J. B. Williams. 2009. Genetic modulation of energy metabolism in birds through mitochondrial function. Proc. R. Soc. Lond. B 276:1685–1693.

Theron, E., K. Hawkins, E. Bermingham, R. E. Ricklefs, N. I. Mundy. 2001. The molecular basis of an avian plumage polymorphism in the wild: a melanocortin-1-receptor point mutation is perfectly associated with the melanic plumage morph of the bananaquit, *Coereba flaveola*. Curr. Biol. 11:550–557.

Tolley, K. A., M. Burge, A. A. Turner, and C. C. Matthee. 2006. Biogeographic patterns and phyogeography of dwaf chamaleons (Bradypodion) in an African biodiversity hotspot. Mol. Ecol. 15:781–793.

Wakeley J, and J. Hey. 1998. Testing speciation models with DNA sequence data. Pp. 157–175 *in* B. Schierwater and R. DeSalle, eds. Molecular approaches to ecology and evolution. Birkhauser-Verlag, Basel, Switzerland.

Waterson, G. A. 1975. On the number of segregating sites in genetical models without recombination. Theoret. Popul. Biol. 7:256–276.

Werger, M. A. 1986. The Karoo and southern Kalahari. Pp. 283–354. *in* M. Evenari, I. Noy-Meir and D. W. Goodall, eds. Hot Deserts and Arid Shrublands. Elsevier, Amsterdam.

Williams, J. B, and B. I. Tieleman. 2005. Physiological adaptation in desert birds. BioScience 55:416–425.

Associate Editor: A. Kopp

Supporting Information

The following supporting information is available for this article:

Table S1. Genetic variation at 11 autosomal microsateliltes genotyped for 285 individuals from 24 populations.

Figure S1. Graphical summary of STRUCTURE results based on 11 autosomal microsatellites using the maximum-likelihood estimate of $k = 2$.

Supporting Information may be found in the online version of this article.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.