Biological Conservation 144 (2011) 2902–2912

Contents lists available at SciVerse ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/biocon

Genetic and morphological divergence in island and mainland birds: Informing conservation priorities

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ARTICLE INFO

Article history:
Received 15 April 2011
Received in revised form 5 August 2011
Accepted 15 August 2011
Available online 9 September 2011

Keywords:
Genetic divergence
Morphological divergence
Evolutionary lineage
Superb fairy-wren
Sex-biased dispersal
Island
Mainland

ABSTRACT

Evolutionary processes can complicate conservation efforts for species with uncertain taxonomic classifications and discrete geographic populations. Discordant morphological and genetic patterns across the geographic range of species further calls for the identification of evolutionary significant units for conservation. Using island and mainland populations of a small Australian passerine (the superb fairy-wren, *Malurus cyaneus*), we examine the relationship between morphological and genetic divergence among two subspecies, *M. c. ashbyi* (Kangaroo Island, South Australia) and *M. c. leggei* (South Australia, mainland), using eight microsatellite markers. Island birds showed clear evidence for morphological divergence, with a larger body size and thinner bill compared to mainland birds. Two genetic clusters were found using Bayesian methods, comprising mainland and island regions. Estimates of recent migration rates between all sites were very low (<2%). Morphological and genetic differentiation between island and mainland sites correlated significantly, but not when controlling for isolation by distance. Genetic and morphological substructure was evident with three distinct genetic clusters in each region. Males, the highly sedentary sex, appeared to drive correlations between morphological and genetic differentiation. Our study provides evidence that the subspecies classification of *M. cyaneus* in island and mainland regions encapsulates two independently diverging populations that can be recognised in conservation planning.

1. Introduction

Populations may show adaptive divergence from each other in geographic isolation if selection pressure is high (Mayr, 1965; Ford, 1974), which has been clearly demonstrated in several cases involving island species (MacArthur and Wilson, 1967; Grant and Grant, 1998; Ricklefs and Bermingham, 2007). Island populations are frequently found to lack genetic diversity in comparison with mainland populations due to founder effects, genetic bottlenecks or genetic drift (Frankham, 1997; Dudaniec et al., 2008), which can further drive divergence. Various environmental factors may drive adaptive divergence within a species including climatic factors, vegetation structure, food availability and inter- and intraspecific competition (Tonnis et al., 2005; Kleindorfer et al., 2006; Grant and Grant, 2008; Hendry et al., 2009; Myers et al., 2010). Adaptive divergence may be followed by genetic differentiation under differential selection pressures on phenotypes (Mayr, 1965; MacArthur and Wilson, 1967). However, morphological divergence may also occur in the face of gene flow as a result of strong selection (Blondel et al., 2006), even in contiguous populations, resulting in discordant genetic patterns between loci encoding adaptive traits (or closely linked to such loci) and neutral genetic markers (De León et al., 2010).

The subtlety of evolutionary processes can therefore complicate conservation efforts that are based on the subspecific rank (Ryder, 1986; Crandall et al., 2000; Zink, 2004). Populations of birds on islands are frequently assigned formal taxonomic ranks, e.g. species or subspecies, due to morphological differentiation, inferred reproductive or geographic isolation (e.g. Rathburn and Montgomerie, 2003). However, the degree of population connectivity (i.e. genetic) between island subspecies and their mainland counterparts is often unknown, and hence its relationship to morphological

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0006-3207/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biocon.2011.08.007

divergence is also unknown (but see: Mundy et al., 1997; Melo and O'Ryan, 2007). For non-volant species the assumption of geographic isolation, even for continental islands, is likely to be reasonable in most cases, however for birds this assumption should be tested directly. Moreover, islands that are connected periodically to mainland regions through the Plio-Pleistocene glacial cycles due to their location on the continental shelf, may have experienced gene flow with the mainland during the Holocene, depending on the elevation profile and the nature of the habitat on exposed land-bridges (e.g. Tiburón Island, Rojas-Soto et al., 2010).

Native woodland bird species in South Australia are undergoing significant declines (Ford et al., 2001; Ford, 2011; Szabo et al., 2011; Watson, 2011), which calls for better identification of evolutionary significant units within this region (Donnellan et al., 2009; Sunnucks, 2011). One species requiring such an assessment is a small insectivorous passerine, the superb fairy wren (*Malurus cyaneus*), as recent surveys of the Mount Lofty Ranges woodlands in South Australia have highlighted its significant decline (Szabo et al., 2011). *M. cyaneus* is classified into two subspecies in South Australia: *M. c. leggei* on the mainland, and *M. c. ashbyi* on Kangaroo Island, a neighbouring continental island (Schodde and Mason, 1999). Molecular genetic and morphological analyses of South Australian *M. cyaneus* have produced contrasting perspectives on divergence between these mainland and island populations. In an earlier molecular genetic study, Etemadmoghadam (2004) failed to find evidence of divergence in microsatellite allele frequencies and phylogenetic structure of mitochondrial DNA sequences between the two regions. Indeed one particular mitochondrial haplotype was found on Kangaroo Island and across south-eastern Australia, including Tasmania. Contrastingly, Schlotfeldt and Kleindorfer (2006) found significant morphological and ecological divergence between the two regions with island wrens having a larger body size (bill, tarsus and wing length), darker female plumage colouration and a wider foraging niche compared with mainland birds. Other biological data are consistent with the morphological perspective. No mark-recapture studies have recorded movement of *M. cyaneus* between Kangaroo Island and the adjacent mainland (Paton et al., 2002). In *M. cyaneus*, dispersal is limited and female-biased, with extra-pair fertilisations being important facilitators of gene flow (Double et al., 2005). Thus, while the mitochondrial data likely rule out species level divergence between the island and mainland populations under a phylogenetic species concept, this island–mainland system offers the opportunity to determine whether morphological variation in *M. cyaneus* is correlated with genetic population structure. Therefore, we investigate whether these populations show evidence of adaptive divergence that warrants both conservation and taxonomic recognition.

Here, we test the predictions that (1) genetic differentiation will be high between morphologically dissimilar island and mainland populations, with little gene flow and low migration rates, (2) males will drive patterns of morphological and genetic structure as they are highly sedentary, while females are the dispersing sex, and (3) when excluding isolation by distance (IBD) effects, morphological differentiation will correlate with genetic differentiation within and between island and mainland populations. Our estimates of population structure and divergence are based on an improved and more comprehensive data set derived from a suite of eight microsatellite loci, larger sample sizes and more detailed geographic sampling in comparison with Etemadmoghadam (2004), which was limited in all sampling aspects. Furthermore the molecular genetic data were collected from the same individuals used in our morphological analyses, which we also expand to improve sampling of within region variability.

2. Materials and methods

2.1. Study species and location

The superb fairy-wren (*M. cyaneus*) is a small (~10 g) Australian passerine that is socially monogamous with group-living cooperative breeders (Rowley, 1965; Rowley and Russell, 1997). Schodde and Mason (1999) recognised six subspecies of *M. cyaneus* based on plumage pattern, colour and length variation in the tarsus, a heritable trait (see Alatalo and Lundberg, 1986). Males are generally philopatric to their natal territory (mean size: 0.6–8.6 ha) whereas females disperse in the first year of life, moving between 5.6–16.9 territories (Mulder, 1995). We focused on two subspecies of *M. cyaneus*: the Kangaroo Island population, *M. c. ashbyi*, and the South Australian mainland population, *M. c. leggei*, whose range includes the Eyre and York Peninsulas, the Mount Lofty Ranges through to Victoria, with a zone of intergradation in western Victoria with the eastern mainland subspecies *M. c. cyanochlamys* (Schodde and Mason, 1999). The three remaining subspecies are found on Bass Strait islands and Tasmania. Kangaroo Island is a large (4500 km^2) continental island that became situated ~14 km from the coast of South Australia due to rises in sea level approximately 7500 to 8900 years ago (Belperio and Flint, 1999; Davies et al., 2002). The island has a slightly warmer and more humid climate with lower precipitation, less ground cover and increased shrub cover than the predominantly forested mainland (Schlotfeldt and Kleindorfer, 2006; Colombelli-Négrel et al., 2009; Colombelli-Négrel and Kleindorfer, 2010).

Adult birds were sampled from four mainland and three island sites. Mainland sites were located in the Mount Lofty Ranges of South Australia, while the mainland subspecies' range also extends to the York and Eyre Peninsulas of South Australia. Three mainland and two island sites have been described previously (Schlotfeldt and Kleindorfer, 2006) and we include an additional mainland site (Newland Head Conservation Park, 35°37′S, 138°29′E) and island site (Parndana Conservation Park, 35°45′S, 137°19′E). Birds were captured in mist-nets over a 2–4 km^2 area within each site. Sites were randomly chosen within seven reserves: (M1) Sandy Creek Conservation Park (1.4 km^2), (M2) Scott Creek Conservation Park (7.1 km^2), (M3) Scott Conservation Park (2 km^2), (M4) Newland Head Conservation Park (10.4 km), (I5) Pelican Lagoon Conservation Park (4.3 km^2), (I6) Parndana Conservation Park (3.1 km^2), and (I7) Flinders Chase National Park (Fig. 1) (300 km^2), where 'M' represents a mainland and 'I' represents an island site.

2.2. DNA extraction

In total, 296 samples were collected for DNA extraction, ranging from 26 to 70 adult individuals per site (Appendix A). Blood samples (5 μl) were collected from the jugular vein of each bird and stored on FTA® card (Smith and Burgoyne, 2004). DNA was extracted from blood following a variation of method #4 for nucleated erythrocytes for use in polymerase chain reaction (PCR) (Smith and Burgoyne, 2004). Discs of 1 mm^2 were cut from the FTA card, washed for 30 min in 500 μl of FTA wash solution (100 mM Tris, 0.1% SDS), then washed twice for 10 min in 500 μl of DNAzol® (Life Technologies, www.lifetech.com), with two final 10-min washes in 500 μl of water. Samples were dried and eluted in TE buffer.

2.3. Microsatellite amplification and genotyping

Ten polymorphic microsatellite loci were amplified for all 296 adult birds (mainland, $n = 148$; island, $n = 148$; Appendices A and C) using primers previously developed for *M. cyaneus* (*Mcyu1*,