Saving Darwin's muse: evolutionary genetics for the recovery of the Floreana mockingbird

Paquita E. A. Hoeck^{1,*}, Mark A. Beaumont²,
Karen E. James³, Rosemary B. Grant⁴,
Peter R. Grant⁴ and Lukas F. Keller¹

¹Zoological Museum, University of Zurich, Winterthurerstrasse 190,
8057 Zurich, Switzerland

²School of Biological Sciences, University of Reading,
Reading RG6 6BX, UK

³Department of Botany, Natural History Museum, Cromwell Road,
London SW7 5BD, UK

⁴Department of Ecology and Evolutionary Biology, Princeton University,
Princeton, NJ 08544, USA

*Author for correspondence (paquitaehoeck@access.uzh.ch).

The distribution of mockingbird species among the Galápagos Islands prompted Charles Darwin to question, for the first time in writing, the 'stability of species'. Some 50 years after Darwin's visit, however, the endemic Floreana mockingbird (*Mimus trifasciatus*) had become extinct on Floreana Island and, today, only two small populations survive on two satellite islets. As Darwin noted, rarity often precedes extinction. To avert extinction, plans are being developed to reintroduce *M. trifasciatus* to Floreana. Here, we integrate evolutionary thinking and conservation practice using coalescent analyses and genetic data from contemporary and museum samples, including two collected by Darwin and Robert Fitzroy on Floreana in 1835. Our microsatellite results show substantial differentiation between the two extant populations, but our coalescence-based modelling does not indicate long, independent evolutionary histories. One of the populations is highly inbred, but both harbour unique alleles present on Floreana in 1835, suggesting that birds from both islets should be used to establish a single, mixed population on Floreana. Thus, Darwin's mockingbird specimens not only revealed to him a level of variation that suggested speciation following geographical isolation but also, more than 170 years later, return important information to their place of origin for the conservation of their conspecifics.

Keywords: museum specimens; genetic diversity; conservation; Galápagos; *Nesomimus*

Rarity, as geology tells us, is the precursor to extinction. We can, also, see that any form represented by few individuals will, during fluctuations in the seasons or in the number of its enemies, run a good chance of utter extinction.

(Darwin 1859, p. 109)

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2009.0778> or via <http://rsbl.royalsocietypublishing.org>.

1. INTRODUCTION

When Charles Darwin visited Galápagos in 1835, he observed and collected specimens of three different mockingbird species (Gould 1837). Darwin noticed the Floreana mockingbird's evident difference from the specimens he collected on other islands. Thus, the former holds a special claim on being a first vital clue for Darwin's theory of speciation under natural selection (Darwin 1839; Steinheimer 2004). The Floreana mockingbird became extinct on Floreana some 50 years after Darwin's visit, most likely owing to introduced predators such as black rats (Curry 1986) and other habitat alterations following human colonization (Charles Darwin Foundation 2008). Today, this endemic species is critically endangered and only occurs on Floreana's satellite islets Champion (20–53 individuals; Grant *et al.* 2000) and Gardner-by-Floreana (200–500 birds; P. E. A. Hoeck & L. F. Keller 2009, unpublished census data; figure 1).

As Darwin (1859) noted, rarity often precedes extinction. To avert extinction, a recovery plan has been drafted to restore natural habitat on Floreana (Charles Darwin Foundation 2009) and to reintroduce *Mimus trifasciatus* (Charles Darwin Foundation 2008). Owing to isolation since the extinction of the geographically interjacent Floreana population, Grant *et al.* (2000) predicted genetic differentiation and loss of genetic variation in the two remnant populations (figure 1). Therefore, a key question raised by the recovery plan is whether the two satellite populations have been evolving independently since well before the human-induced decline of the Floreana population.

Here we show that coalescent-based models combined with genetic data from contemporary samples and museum specimens—including two collected by Darwin and Fitzroy on Floreana—can be used to estimate divergence times and inform the reintroduction strategy.

2. MATERIAL AND METHODS

(a) Sample collection

We obtained blood samples from the Champion and Gardner populations in 2006–2008 ('2008 samples'; figure 1) and small toe-pad samples from specimens collected on both satellite islets in 1905/1906 ('1906 samples'; figure 1) by a California Academy of Sciences (CAS) expedition. We also analysed two specimens from Floreana itself collected in 1835 ('Floreana specimens'; figure 1) and held at the Natural History Museum (NHM, London, UK).

(b) Genotyping

Seventeen microsatellite loci were amplified from all samples as described elsewhere (Hoeck *et al.* 2009), 11 of which displayed polymorphism. Contemporary samples were genotyped once, except in the case of amplification failure, in which case PCR was repeated. CAS historic samples were genotyped four times at each locus (Hoeck *et al.* in press). Genotyping of the two Floreana specimens was verified by repeating the fragment analysis eight to 16 times per sample and by control amplifications at the NHM. Genotyping error rates per locus and consensus genotypes were determined using GIMLET (Valiere 2002) and results crosschecked by hand. Genotyping details and success rates are described in the electronic supplementary material.

(c) Genetic diversity and differentiation

Genotypic and Hardy–Weinberg equilibrium were tested as described in the electronic supplementary material. Genetic diversity within populations was measured as number of alleles, allelic richness, expected heterozygosity and number of private alleles. Differentiation between contemporary and historic populations was estimated using Nei's D_s (Langella 2000) and F_{ST} (Goudet 2001). An analysis of molecular variance (AMOVA) was conducted using ARLEQUIN v. 3.11 (Excoffier *et al.* 2005).

Figure 1. Floreana (17 253 ha) and its satellite islets Champion (9.4 ha, less than 1 km away) and Gardner (81 ha, approx. 8 km from Floreana). Number of samples (*n*) obtained from each island and time period and estimates of genetic diversity (NA, number of alleles; AR, allelic richness; He, expected heterozygosity). Scale bar, 10 km.

(d) Coalescent modelling

The genetic data were analysed in a Bayesian framework using a coalescent-based model. No methods currently exist that can incorporate historic and current samples in a model with divergent populations. Here, based on the model of Beaumont (2003), we developed a new approach that can, in principle, accommodate any number of populations and branching topologies (see the electronic supplementary material for details). We modelled populations diverging from a common ancestral population in the framework introduced by Beaumont (2003) to compute Bayesian posterior distributions for demographic parameters using temporally sampled genetic data (figure 2a; Beaumont 2003). We performed the analysis with two sets of priors (see the electronic supplementary material for details). One analysis was performed with narrower, gamma-distributed, priors on N_e , based on census information from Champion and Gardner (Grant *et al.* 2000; P. E. A. Hoeck & L. F. Keller 2009, unpublished census data), and the other analysis assumed that the prior on N_e was uniform between 0 and 10 000 for all islands. The priors used for generation time and times of divergence were the same for either analysis, and were uniform distributions with upper and lower bounds based on reasoned guesses as to minimum and maximum possible values (electronic supplementary material). The commented C code for the model is available at http://www.rubic.reading.ac.uk/~mab/stuff/mock_analysis.zip.

We compared two scenarios of divergence: one in which the Gardner population first started to diverge from the common ancestral population, followed by divergence between Floreana and Champion (topology 1, figure 2a), and another in which Champion first diverged (topology 2). We used genetic data from a total of six sampling occasions for our analyses (electronic supplementary material).

3. RESULTS AND DISCUSSION

The contemporary Champion and Gardner populations are clearly divergent (pairwise $F_{ST} = 0.533$, $p < 0.005$; $D_s = 0.26$) owing, in part, to the low genetic diversity observed on Champion and pronounced genetic drift over the last century (figure 1). Between 1906 and 2008 Champion lost 39 per cent of its expected heterozygosity, as predicted theoretically by Grant *et al.* (2000) based on observed population sizes, whereas Gardner lost only 6 per cent (figure 1). The extent of genetic drift over the last century is exemplified by the fact that an allele with a

frequency as high as 0.64 in the Champion population in 1906 was completely lost by 2008. Although genetic drift since 1906 contributed somewhat to the divergence of the two satellite populations in 2008 (pairwise F_{ST} increased by 0.06 from 1906 to 2008), the two populations were already considerably differentiated in 1906 ($F_{ST} = 0.47$, $p < 0.005$; $D_s = 0.23$). This finding was corroborated by an AMOVA, in which 46 per cent of the genetic variation was found among islands, 6 per cent between the two time periods within Champion and Gardner, respectively, and 48 per cent within time period and population (p -values for all comparisons less than 0.001).

Is the divergence in 1906 evidence that the two satellite populations have been evolving independently since long before the human-induced decline of the interjacent population on Floreana, or does it reflect an evolutionary young phenomenon related to that decline? Our coalescent analyses suggest that the Gardner population started to diverge from the Floreana population before the Champion population did (the posterior probability of topology 1 was 0.83). This is not surprising given the closer geographical proximity of Champion and Floreana (figure 1) and the south–southeast direction of prevailing winds that could act as a deterrent to immigration from Floreana to Gardner. The estimates of divergence times were well bounded and suggest a relatively recent divergence (figure 2b,c). Under topology 1 (Gardner diverged first; figure 2a), we estimated a mode of approximately 270 years ago for the younger divergence of Champion (figure 2b) and a mode of 450 years ago for the older divergence of Gardner (figure 2c). Results under topology 2 (Champion diverged first) differed somewhat, but not fundamentally (figure 2b,c). Under any scenario, it is highly unlikely that any divergence began more than 800 years ago (figure 2c).

Figure 2. (a) The two topologies of population divergence investigated with the coalescent models with the Gardner (topology 1) or the Champion (topology 2) population diverging first. (b,c) Posterior probability distributions of the divergence times for Champion and Gardner under the two topologies. Solid lines, topology 1; dashed lines, topology 2. (d,e) Posterior probability distribution of the current effective population size (N_e) on Champion and Gardner, respectively. Current N_e values refer to the harmonic mean N_e between 1906 and 2008. The dotted lines represent the informative prior, based on the information from the census population sizes. Solid lines, topology 1; dashed lines, topology 2. (f) Total number of alleles across all loci in the contemporary Champion and Gardner populations, and in the two Floreana specimens. Grey: alleles that occurred in both contemporary populations and in the Floreana specimens. Alleles today only found on Gardner (dotted) or Champion (hatched), or only detected in the Floreana specimens (black).

Our coalescent analyses, therefore, do not support the hypothesis that this divergence is ancient. Rather, they are compatible with a model of three somewhat subdivided populations connected by recurrent gene flow. With the decline and ultimate extinction of the Floreana population, gene flow declined, as indicated by the private alleles, some of them with frequencies as high as 1, in both Gardner and Champion in 2008 (figure 2f). A former connection through gene flow is further supported by the finding that the two Floreana specimens shared 16 (50%) of their alleles with both contemporary populations, but also harboured

alleles that today are private to either one of the satellite populations (figure 2f). Thus, part of the variation once present on Floreana has persisted in both satellite populations. Our results therefore suggest that current and future management actions should focus on conserving the two satellite populations *in situ* and establishing a single third population on Floreana using birds from both islets to maximize genetic diversity upon which selection can act. This view is further supported by evidence from another avian radiation in Galápagos, Darwin's finches, where interbreeding of species has increased the additive genetic variance in

morphological traits under selection, which may facilitate evolution along novel trajectories (Grant & Grant 2008). Nothing could better prepare a reintroduced population for the future. The short divergence times between the Gardner and Champion population do not imply that no evolutionary changes have occurred or that individuals are not locally adapted. Instead, they imply that mixing of the two populations will probably lead to heterosis in addition to increased additive genetic variance. Theory-based research suggests that this is likely when divergence levels are relatively high and effective population sizes relatively low (Whitlock *et al.* 2000) as is the case in the Floreana mockingbird (figure 2d,e).

All fieldwork and procedures were approved by the Galápagos National Park Service.

We thank the Charles Darwin Research Station, especially Felipe Cruz, the Galápagos National Park Service, TAME airlines, the Bird Group of the Natural History Museum, especially Mark Adams, Joanne Cooper and Robert Prys-Jones, the California Academy of Sciences, especially Maureen Flannery and John Dumbacher, and all field assistants. Thanks to Darwin and Fitzroy for collecting the Floreana specimens and various colleagues for helpful discussions, especially Randal Keynes. This research was funded by the Forschungskredit of the University of Zurich, the Balzan Foundation and the Basler Stiftung für biologische Forschung.

- Beaumont, M. A. 2003 Estimation of population growth or decline in genetically monitored populations. *Genetics* **164**, 1139–1160.
- Charles Darwin Foundation 2008 *The reintroduction of the Floreana mockingbird to its island of origin*. Puerto Ayora, Galapagos: Charles Darwin Foundation and Galapagos National Park Service.
- Charles Darwin Foundation 2009 *Project Floreana 2009–2013: an integrated approach*. Puerto Ayora, Galapagos: Charles Darwin Foundation.
- Curry, R. L. 1986 Whatever happened to the Floreana mockingbird? *Noticias de Galapagos* **43**, 13–15.
- Darwin, C. R. 1839 *Narrative of the surveying voyages of His Majesty's Ships Adventure and Beagle between the years 1826 and 1836, describing their examination of the southern*

shores of South America, and the Beagle's circumnavigation of the globe. Journal and remarks 1832–1836. London, UK: Henry Colburn.

- Darwin, C. R. 1859 *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. London, UK: John Murray.
- Excoffier, L., Laval, G. & Schneider, S. 2005 ARLEQUIN v. 3.0: an integrated software package for population genetics data analysis. *Evol. Bioinform. Online* **1**, 47–50.
- Goudet, J. 2001 *FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3)*. Lausanne, Switzerland: Lausanne University.
- Gould, J. 1837 Three species of the genus *Orpheus*, from the Galapagos, in the collection of Mr Darwin. *Proc. Zool. Soc. Lond.* **5**, 27.
- Grant, P. R. & Grant, B. R. 2008 *How and why species multiply. The radiation of Darwin's finches*. Princeton, NJ: Princeton University Press.
- Grant, P. R., Curry, R. L. & Grant, B. R. 2000 A remnant population of the Floreana mockingbird on Champion island, Galapagos. *Biol. Conserv.* **92**, 285–290. (doi:10.1016/S0006-3207(99)00092-0)
- Hoeck, P. E. A., Bollmer, J. L., Parker, P. G. & Keller, L. F. In press. Differentiation with drift: a spatio-temporal genetic analysis of Galapagos mockingbird populations (*Mimus* spp.). *Phil. Trans. R. Soc. B*.
- Hoeck, P. E. A., Bucher, T. B., Wandeler, P. & Keller, L. F. 2009 Microsatellite primers for the four Galápagos mockingbird species (*Mimus parvulus*, *M. macdonaldi*, *M. melanotis*, and *M. trifasciatus*). *Mol. Ecol. Resour.* **9**, 1538–1541.
- Langella, O. 2000 Populations 1.2.30. Laboratoire Populations, Génétique et Evolution. Centre National de la Recherche Scientifique, CNRS. UPR9034, Gif Sur Yvette. See <http://bioinformatics.org/~tryphon/populations/>.
- Steinheimer, F. D. 2004 Charles Darwin's bird collection and ornithological knowledge during the voyage of H.M.S. 'Beagle', 1831–1836. *J. Ornithol.* **145**, 300–320. (doi:10.1007/s10336-004-0043-8)
- Valiere, N. 2002 GIMLET: a computer program for analysing genetic individual identification data. *Mol. Ecol. Notes* **2**, 377–379. (doi:10.1046/j.1471-8286.2002.00228.x)
- Whitlock, M. C., Ingvarsson, P. K. & Hatfield, T. 2000 Local drift load and the heterosis of interconnected populations. *Heredity* **84**, 452–457. (doi:10.1046/j.1365-2540.2000.00693.x)