



This is a repository copy of *Genetic structure among Charadrius plovers on the African mainland and islands of Madagascar and St Helena.*

White Rose Research Online URL for this paper:
<http://eprints.whiterose.ac.uk/139203/>

Version: Accepted Version

Article:

Remedios, N.A., Küpper, C., Székely, T. et al. (4 more authors) (2018) Genetic structure among Charadrius plovers on the African mainland and islands of Madagascar and St Helena. *Ibis*. ISSN 0019-1019

<https://doi.org/10.1111/ibi.12694>

This is the peer reviewed version of the following article: dos Remedios, N. , Küpper, C. , Székely, T. , Zefania, S. , Burns, F. , Bolton, M. and Lee, P. L. (2018), Genetic structure among Charadrius plovers on the African mainland and islands of Madagascar and St Helena. *Ibis*, which has been published in final form at <https://doi.org/10.1111/ibi.12694>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



eprints@whiterose.ac.uk
<https://eprints.whiterose.ac.uk/>

DR. NATALIE DOS REMEDIOS (Orcid ID : 0000-0002-5412-4039)

DR. MARK BOLTON (Orcid ID : 0000-0001-5605-687X)

Article type : Original Paper

Editor: Jon Martin Collinson

Running head: African plover population genetics

Genetic structure among Charadrius plovers on the African mainland and islands of Madagascar and St Helena

NATALIE DOS REMEDIOS,^{1,2*} CLEMENS KÜPPER,³ TAMÁS SZÉKELY,¹ SAMA ZEFANIA,⁴ FIONA BURNS,⁵ MARK BOLTON⁵ & PATRICIA L. M. LEE⁶

¹Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath, BA2 7AY, UK

²Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield S10 2TN,
UK

³Max Planck Institute for Ornithology, Eberhard Gwinner Str., 82319 Seewiesen, Germany

⁴Institut Supérieur de technologie de Menabe Morondava, Faculty of Sciences, University of Toliara,
Madagascar

⁵RSPB Centre for Conservation Science, The Lodge, Sandy, Bedfordshire, SG19 2DL, UK

⁶Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University,
Warrnambool Campus, Princes Hwy, Warrnambool, VIC 3280, Australia

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ibi.12694

This article is protected by copyright. All rights reserved.

*Corresponding author.

E-mail: n.dos-remedios@sheffield.ac.uk

Colonisation of islands by long distance dispersers has great impact on genetic diversification among populations and may spearhead speciation events. We investigated intra- and interspecific divergence in Charadrius plovers with populations on mainland Africa, Madagascar and St Helena. We analysed microsatellite loci and sequence data from four nuclear and two mitochondrial gene regions.

Charadrius plovers are shorebirds with high dispersal and mobility. Our results confirmed genetic differentiation between Madagascar and mainland populations of three plover species (White-fronted Plovers, *C. marginatus*; Kittlitz's Plovers, *C. pecuarius*; and based on sequence data only, Three-banded Plovers, *C. tricollaris*) but highlight substantial variation in levels of intraspecific divergence among the three species. Namely, the Kittlitz's Plover, a dispersive habitat generalist with a polygamous mating system, exhibited lower island-mainland differentiation (0.05% COI sequence divergence) than two monogamous species, the White-fronted Plover (0.6% COI divergence) and Three-banded Plover (1.6% COI divergence). In addition, past colonisation of the islands of St Helena and Madagascar by ancestors of today's Kittlitz's Plover led to the evolution of two endemic island species, the Madagascar Plover *C. thoracicus* and the more closely-related St Helena Plover *C. sanctaehelena*. We discuss the factors driving species differences in island-mainland divergence, and highlight the importance of conserving genetically unique island populations and island habitats to safe-guard future evolutionary potential.

Keywords: dispersal, population genetics, speciation, waders

Islands have long been known to have a profound impact on the formation and diversity of species, a point that inspired Darwin (1845) on the mechanisms of evolution and led to the emergence of an entire discipline: island biogeography (MacArthur & Wilson 1967, Warren et al. 2015). Islands are

particularly important in speciation for highly mobile species such as birds, for which mainland habitats often provide no barriers to dispersal. Mainland populations of these species remain highly connected and genetically homogenous meaning that the emergence of genetic differentiation, the precursor of speciation, is hindered (Ibrahim et al. 1996, Phillimore et al. 2006, Agnarsson & Kuntner 2012). For example, mainland populations of Kentish Plover *Charadrius alexandrinus* across continental Eurasia are genetically similar up to 10 000 km apart, with divergence observed only among populations isolated by oceanic barriers (Küpper et al. 2012). Furthermore, populations that successfully colonise smaller, more distant islands are expected to develop greater genetic divergence from mainland populations than those occupying larger islands, closer to the mainland (Amos & Harwood 1998).

To further investigate the role of islands in promoting genetic differentiation and speciation in avian populations, we focused on the small plovers, genus *Charadrius* (c. 31 species; Linnaeus 1758). Plovers are shorebirds capable of traversing large distances, and several species are migratory (Stenzel et al. 1994, Hedenström et al. 2013). They have precocial offspring and are of interest in evolutionary biology for their highly variable mating systems (Thomas et al. 2007). Nine *Charadrius* species breed in Africa including three species with populations on both Madagascar and the mainland. Previously we assigned these African species to three distinct phylogenetic clades (see Fig. 1a) that independently colonised the continent following dispersal southwards from Palearctic, Saharo-Arabian and Central Asian regions (dos Remedios et al. 2015). Each of the three clades includes one species with populations on both Madagascar and the mainland: the Three-banded Plover *C. tricollaris* (Clade a); the Kittlitz's Plover *C. pecuarius* (Clade e); and the White-fronted Plover *C. marginatus* (Clade f; see Fig. 1 for species distributions). Levels of genetic divergence among these populations have not previously been examined and comparison between species may reveal insights on the development of genetic differentiation, and ultimately speciation.

Among the African small plovers are two island endemics, one species on the island of St Helena and the other on Madagascar: the St Helena Plover *C. sanctaehelenae* and Madagascar Plover *C. thoracicus* (Hayman et al. 1986). Madagascar is a large island, 587 040 km² in size, separated from mainland Africa by 400 km; whereas at 420 km², St Helena is a much smaller and far more isolated

island, lying 1 950 km from mainland Africa. The St Helena Plover and Madagascar Plover are close sister species to the widespread Kittlitz's Plover, representing two of the most recent cases of speciation within the genus *Charadrius* (dos Remedios et al. 2015). The St Helena Plover is approximately 30% larger than the Kittlitz's Plover and exhibits reduced tan colouration on the breast whilst the Madagascar Plover is distinguished from both sister species by a strong black breast band (del Hoyo et al. 1996, Rowlands et al. 1998). The conservation status of both endemics is 'vulnerable' and populations are at risk from habitat loss as well as introduced predators such as cats and rats (Birdlife International 2016a, 2016b). Until recently, the St Helena Plover population was in decline and classified as 'critically endangered' (McCulloch 2009), but successful management of its grassland breeding areas has prompted an increase in population size, from approximately 220 adults in 2006 to approximately 560 in 2016 (Birdlife International 2016a). The Madagascar Plover has a larger population of approximately 2000 individuals, but this population is currently thought to be in decline due to the destruction of habitats on the west coast of Madagascar (Birdlife International 2016b). Both species are habitat specialists, breeding in areas of short-sward grassland, making them particularly vulnerable to habitat loss.

In this study, we first examined population level genetic divergence among African *Charadrius* plovers, with a focus on island-mainland differentiation, in three species with breeding populations on both mainland Africa and Madagascar. For two species, the Kittlitz's Plover and the White-fronted Plover, we assessed population divergence using both microsatellite data and sequence-based (nuclear and mitochondrial) analyses. For a third species, the Three-banded Plover, microsatellite analyses were not possible due to a limited samples size on mainland Africa (Kenya, $n = 1$), therefore we conducted sequence-based analysis only. We predicted that the connectivity of plover populations would be disrupted by oceanic barriers, and hence island populations should be clearly genetically differentiated from their mainland counterparts whereas high connectivity among continental populations should lead to lower genetic differentiation.

Additionally, we used microsatellite and sequence-based analyses to examine genetic divergence between populations of the Kittlitz's Plover and its two closely related endemic sister species, the St Helena Plover and Madagascar Plover. Based on island size, population size and

distance to the mainland, we expected that Kittlitz's Plovers exhibit a greater degree of divergence from St Helena Plovers than from Madagascar Plovers. Lastly, for all populations assessed using microsatellite data, we evaluated coefficients of inbreeding, with the expectation that levels of inbreeding would be greater for island than mainland populations.

METHODS

Taxon sampling and DNA extraction

Samples were collected from five plover species (sample sizes listed in Table 1; distribution maps in Fig. 1). For each sample, 25–50 μ l of blood was collected and stored in 1 ml Queen's Lysis Buffer (Seutin et al. 1991) or 1 ml 95% ethanol. DNA was extracted from blood samples using an ammonium acetate precipitation method (Nicholls et al. 2000, Richardson et al. 2001).

Genotyping at microsatellite loci was carried out for Madagascar and mainland African populations of two African endemic species, the White-fronted Plover and Kittlitz's Plover, as well as for two close sister species of the latter, the Madagascar Plover and St Helena Plover (Table 1). Analyses of sequence data were performed for all species and sampling sites, including both Madagascar and mainland populations of the White-fronted Plover, Kittlitz's Plover and Three-banded Plover (Table 1).

Microsatellite genotyping

Utilising microsatellite primers largely developed for the genus *Charadrius* (see Table S2 and S3), we initially tested markers in eight Kittlitz's Plover and eight White-fronted Plover samples. We selected polymorphic loci for which at least two different alleles were detected and designed multiplexes using Multiplex Manager v1.2 (Holleley & Geerts 2009) for these two species separately. Multiplexes designed based on Kittlitz's Plover samples were also used for analyses of its sister species, the St Helena Plover and Madagascar Plover. We conducted polymerase chain reactions (PCRs) in 2 μ l reactions, including each primer at 0.2 μ M (forward primers labelled with fluorescent dye) and 1 μ l Qiagen Multiplex PCR MasterMix. Each reaction was sealed with mineral oil to prevent evaporation.

PCR amplification was conducted on a DNA Engine Tetrad 2 Peltier Thermal Cycler (Bio-Rad, Hercules, USA) as follows: 95 °C for 15 min, 35 cycles of 94 °C for 30 s, 56 °C for 90 s, 72 °C for 60 s, and a final extension of 60 °C for 30 min. Amplicons were visualised on an ABI 3730 automated DNA analyser and fragment lengths were scored using GeneMapper software version 4.1 (Applied Biosystems, Waltham, USA). For 5% of samples, PCR amplification and scoring were repeated a second time in a blind test. Genotyping was consistent across replicates in all cases.

Sampled individuals in each population were putatively unrelated with known parent-offspring and sibling pairs excluded. To further avoid inadvertent sampling of highly related individuals, levels of genetic relatedness among individuals were evaluated in MLRelate (Kalinowski et al. 2006) and those with relatedness of $R > 0.5$ (corresponding to full sibling or parent-offspring relatedness; Blouin 2003) were removed from further analyses. Removed samples included 19 out of 82 White-fronted Plovers, 32 of 112 Kittlitz's Plovers, 20 of 31 St Helena Plovers and 13 of 31 Madagascar Plovers (for final sample sizes per population see Table 1).

Deviations from Hardy-Weinberg equilibrium at each marker were assessed in Cervus v3.0.3 (Kalinowski et al. 2007) and markers were excluded from further analyses where deviations occurred and null allele frequencies were high (>0.2) in two or more populations. For Kittlitz's Plovers, St Helena Plovers and Madagascar Plovers, nine markers were included in the final set; for White-fronted Plovers, 17 markers remained (see marker sets in Tables S2 and S3).

Microsatellite-based population analyses

To determine relative levels of genetic diversity for each marker and species, allelic richness and inbreeding coefficients (F_{IS}) were estimated in FSTAT 2.9.3 (Goudet 1995), and global fixation indices (F_{ST} , G'_{ST} and D) per species were estimated using the 'diveRsity' package in R ('diff-Calc' function; Keenan et al. 2013). To evaluate genetic differentiation among populations of Kittlitz's Plovers and White-fronted Plovers, Arlequin 3.5.2 (Excoffier & Lischer 2010) was used to estimate pairwise fixation indices (F_{ST}) and perform Analysis of Molecular Variance (AMOVA). Using

AMOVA, we assessed all possible population groupings of these two species to determine which arrangement maximised molecular variance between as opposed to within groups.

We additionally assessed genetic structure among populations using Principal Component Analysis (PCA; 'adegenet' package in R (v3.0.3; R Core Team 2013)) and STRUCTURE 2.3.4 (Pritchard et al. 2000, Hubisz et al. 2009). Both programs were implemented for: the White-fronted Plover; the Kittlitz's Plover alongside the St Helena Plover and Madagascar Plover; and the Kittlitz's Plover alone. STRUCTURE analyses were carried out to determine the number of genetic clusters (K) within each sample set. An admixture model was utilised, with allele frequencies independent, for 500 000 MCMC (Markov chain Monte Carlo) generations after a burn-in of 100 000. K values of 1 to 4 were assessed for the White-fronted Plover; K values of 1 to 7 for the Kittlitz's Plover, St Helena Plover and Madagascar Plover; and K values of 1 to 5 for the Kittlitz's Plover only analyses. To determine the convergence of MCMC runs, convergence of the parameter alpha (indicating relative admixture between populations) was assessed throughout each run. STRUCTURE analyses were repeated twice for each dataset and results (and alpha convergence) were consistent between repeats. Results were evaluated using Structure Harvester (Earl & vonHoldt 2012) and plots were constructed with CLUMPP (Jakobsson & Rosenberg 2007) and DISTRUCT (Rosenberg 2004).

Sequence analyses

Six genes were targeted for PCR amplification and sequencing including four nuclear loci: ADH5 (alcohol dehydrogenase 5; 829 bp partial coding sequence; Fain et al. 2007), FGB7 (β -fibrinogen intron 7; 840 bp; Prychitko & Moore 1997), MYO2 (myoglobin intron 2; 688 bp; Slade et al. 1993) and RAG1 (recombination activating gene 1; 911 bp partial coding sequence; Groth & Barrowclough 1999); and two mitochondrial loci: COI (cytochrome oxidase I; 626 bp; Hebert et al. 2004) and ND3 (NADH dehydrogenase subunit 3; 401 bp; Chesser 1999). Sequences for one population per species were the same as those utilised by dos Remedios et al. (2015) (see Table S1 for details). For full details of primers, PCR and sequencing conditions see dos Remedios et al. (2017).

Sequences were edited and aligned in CodonCode Aligner 3.7.1 (CodonCode Corporation, Centerville, USA) using the ClustalW algorithm, and sequence data were deposited in the GenBank sequence database (for accession numbers see Table S1). DNAsp v5 (Librado & Rozas 2009) was used to assess nucleotide divergence across sampled populations.

RESULTS

Population level island-mainland genetic differentiation

Clear genetic differentiation was present between Madagascar and mainland Africa for all three species assessed at the population level, though the degree of differentiation varied among species.

Specifically, populations of White-fronted Plovers and Three-banded Plovers exhibited greater island-mainland differentiation than populations of Kittlitz's Plovers (as described below). For White-fronted Plovers and Kittlitz's Plovers, this pattern was evident in global fixation indices based on microsatellite data: White-fronted Plover F_{ST} 0.367; Kittlitz's Plover F_{ST} 0.029 (not evaluated in Three-banded Plovers due to low sample size).

White-fronted Plovers

Based on microsatellite (allele size) data, the White-fronted Plovers of Madagascar (two sites) represented a genetic cluster distinct from the Namibian population (3400 km away). 46.13% of microsatellite variance was attributed to differences between these clusters based on AMOVA analysis (Table 2; see Table 3 for pairwise fixation indices, F_{ST}) and these clusters were identified based on both STRUCTURE analyses (Fig. 2a; $K = 2$, Delta $K = 4509.27$ vs. $K = 3$, Delta $K = 0.81$; for full K value comparison see Fig. S1 and Table S4) and PCA (see Fig. 2a).

Madagascar and mainland (Namibian and South African) populations exhibited moderate genetic divergence based on mitochondrial and nuclear sequence data (0.9% mitochondrial divergence; 0.2% nuclear divergence; Table 4) and among mainland (Namibian and South African)

White-fronted Plover populations, genetic differentiation also emerged, though the overall degree of divergence was lower and divergence was present in mitochondrial sequence data only (0.4% mitochondrial divergence; 0% nuclear divergence; Table 4; Table S5).

Kittlitz's Plovers

Kittlitz's Plovers on Madagascar and mainland Africa (Kenya and Senegal) formed two genetic clusters based on microsatellite loci, albeit with weaker differentiation than for White-fronted Plovers. Only 3.73% of molecular variance emerged between Madagascar and mainland populations of the Kittlitz's Plover based on AMOVA (Table 2; see Table 3 for pairwise fixation indices, F_{ST}) and both PCA and STRUCTURE analyses concurred in identifying weak divergence between clusters (Fig. 2b; $K = 2$, Delta $K = 331.94$ vs. $K = 3$, Delta $K = 16.99$; for full K value comparison see Fig. S1 and Table S4). Between the two mainland populations in Kenya and Senegal, there was no identifiable genetic structure in the microsatellite data (Fig. 2b). These results were qualitatively the same whether we included or excluded the two sister species, the St Helena Plover and Madagascar Plover in this analysis (Fig. 2b and 2c).

Based on mitochondrial sequence data, Kittlitz's Plover populations exhibited extremely low diversity between Madagascar and mainland populations (<0.1% divergence; Table 4; Table S5) and also among mainland sites (0.1% mitochondrial divergence). In comparison, at nuclear loci, greater overall sequence diversity was present in terms of the number of polymorphic sites (S ; Table 4; Table S5) yet divergence between populations was again low, with 0.2% nuclear divergence between Madagascar and mainland populations and 0.1% divergence among mainland sites.

Three-banded Plovers

The Three-banded Plovers of Madagascar and Kenya displayed moderate genetic differentiation based on mitochondrial sequence data (1.1% divergence), and low divergence at the four nuclear loci (0.04% divergence; Table 4; Table S5). This species was not assessed at microsatellite loci due to low sample size.

Species divergence on Madagascar and St Helena

Regarding the genetic similarity between the Kittlitz's Plover and its close sister species, the endemic St Helena Plover and Madagascar Plover, the results of microsatellite analyses (based on allele size) were discordant to those of mitochondrial and nuclear sequence-based analyses. Whereas all populations of Kittlitz's Plovers appeared more similar to the Madagascar Plovers than St Helena Plovers based on microsatellite analyses (STRUCTURE and PCA; inter-specific pairwise F_{ST} 0.300 and 0.451 respectively; Fig. 2c, Table 3), Kittlitz's Plover populations appeared more similar to St Helena Plovers than Madagascar Plovers based on sequence data (Table 4).

Inbreeding

Observed levels of heterozygosity (H_o) were lower than expected (H_e) in all study populations (Table 5, Tables S2 and S3) and inbreeding coefficients (F_{IS}) across all sampled populations were positive (Table 5). However, inbreeding coefficients (F_{IS}) among White-fronted Plover populations were not significant after correction for multiple testing (Table 5; adjusted nominal level for multiple comparisons: $p = 0.001$) and among Kittlitz's Plover populations, p-values were significant at only two of the five sites (Andavadoaka in Madagascar and Kenya). Additionally, inbreeding coefficients for St Helena Plovers and Madagascar Plovers were non-significant (Table 5). All inbreeding coefficients remained qualitatively the same when assessed based on full sample sets, including individuals with relatedness of $R > 0.5$.

DISCUSSION

Intra-specific island-mainland genetic differentiation

Populations of three Charadrius species in Madagascar (White-fronted Plovers, Kittlitz's Plovers and Three-banded Plovers) all demonstrated genetic divergence from their mainland African conspecifics. The level of divergence among Kittlitz's Plover populations was lower by an order of magnitude compared to White-fronted Plovers and Three-banded Plovers based on microsatellite loci (allele size) and mitochondrial sequence data. Additionally, Kittlitz's Plovers exhibited extremely low differentiation on the mainland, and across larger distances (Kenya, Namibia, Senegal and South Africa; 6400 km apart) than White-fronted Plovers (Namibia and South Africa; 1200 km apart). The higher genetic connectivity of the Kittlitz's Plover compared with the White-fronted and Three-banded Plovers is consistent with differences in their behaviour and ecology (discussed in further detail below). Yet even for the particularly mobile Kittlitz's Plover, ocean barriers are an isolating factor, with clear genetic divergences from its two endemic island sister species, the Madagascar Plover and St Helena Plover.

White-fronted Plovers exhibited more pronounced genetic differentiation among island-mainland populations than did Kittlitz's Plovers. Given the high genetic structure and phenotypic variation in plumage colouration observed across the White-fronted Plover species range (Hayman et al. 1986), we suggest that the current classification of island and mainland subspecies (as described by Delany et al. 2009) for the White-fronted Plovers of Namibia/ South Africa and Madagascar (*C. marginatus arenaceus* and *C. marginatus tenellus*) may be appropriate. Similarly, we support the subspecies status of the Three-banded Plovers of Kenya and Madagascar (*C. tricollaris tricollaris* and *C. tricollaris bifrontatus*). Based on a limited sample size, we did not find sufficient support for the recent proposal of the latter as two distinct species (del Hoyo et al. 2014). We advise further genetic studies with larger sample sizes to clarify this, as for the *tricollaris* subspecies we were only able to include a single individual, sampled opportunistically in Kenya, in our study.

Species differences in genetic structure

The differences identified in population-level divergence between Kittlitz's Plovers, White-fronted Plovers and Three-banded Plovers on Madagascar and mainland Africa may be due to several causative factors. Populations of Kittlitz's Plovers may exhibit greater genetic similarity due to more recent colonisation of Madagascar, a larger historical effective population size, or higher levels of dispersal and population connectivity. Further evidence is required to determine colonisation dates for these species, as well as historical population trends, though at present, all three species number more than 10 000 mature individuals, across ranges of > 20 000 km² (Birdlife International, 2016c, 2016d, 2016e). Concerning dispersal however, previous evidence does suggest that Kittlitz's Plovers have intrinsically higher levels of dispersal than the other two species (despite their overall physiological similarities) and that this may be linked to differences in habitat specialisation and mating behaviour (Eberhart-Phillips et al. 2015, D'Urban Jackson et al. 2017).

Behaviour and ecology strongly influence the propensity of an organism to disperse away from their natal or breeding population (Greenwood & Harvey 1982, Phillimore et al. 2006), with consequences for population connectivity, gene flow and speciation (Edwards et al. 2005, D'Urban Jackson et al. 2017; Küpper & dos Remedios In press). Kittlitz's Plovers are generalist shorebirds found in areas of dried mud, short grass or sand. They are widely-distributed across Sub-Saharan Africa and are often opportunistic, adapting to novel environments that may not fit their optimal habitat requirements (Cassey 2002). In contrast, White-fronted Plovers are specialists with a preference for sandy areas and Three-banded Plovers favour firm sand, mud or gravel shores for breeding (del Hoyo et al. 1996). Kittlitz's Plovers also differ from the other study species in having a polygamous rather than monogamous mating system (Parra et al. 2014). Monogamous species often exhibit higher levels of philopatry and are more site-faithful, whereas in polygamous species, individuals may travel large distances to find multiple mates in different locations within and between breeding seasons (Reynolds & Cooke 1988, Cézilly et al. 2000, Pearson & Colwell 2013). Such dispersal is likely to increase gene flow between polygamous populations in contrast to monogamous ones (Eberhart-Phillips et al. 2015, D'Urban Jackson et al. 2017). In support of these dispersal differences, data on resightings of monitored individuals in Madagascar indicated greater distances of

dispersal for the Kittlitz's Plover (resighted up to 113 km from capture site) than for the White-fronted Plover (resighted up to 15 km away; Zefania & Székely 2013, Eberhart-Phillips et al. 2015).

The St Helena Plover is currently the only endemic bird species on St Helena, and given the isolation of the island (almost 2000 km from the mainland), dispersal events here from the mainland are likely to be rare. It is therefore worth noting that successful colonisation of this island has occurred once only, by ancestors of the Kittlitz's Plover, the most widely dispersing of the African endemic species. In contrast, Madagascar, a larger island closer to Africa, has been colonised more frequently and is currently home to four plover species (within clades a, e and f; Fig. 1a). Long-distance dispersers may either have highly connected, genetically homogenous populations (Belliere et al. 2000, Claramunt et al. 2012), or increased population divergence due to their propensity to colonise novel habitats (Shaw 1995, Ibrahim et al. 1996, Phillimore et al. 2006). Our results suggest that both cases hold true for the Kittlitz's Plover, with high connectivity across the mainland and stronger genetic differentiation leading to speciation following colonisation of offshore islands.

Species divergence on Madagascar and St Helena

Concerning the two endemic island species, the degree of genetic divergence of the Madagascar Plover and St Helena Plover from the widespread Kittlitz's Plover did not differ significantly from the observed intra-specific variation of the White-fronted Plover and Three-banded Plover. The endemic island species do exhibit strong phenotypic differences in both plumage colouration and body size however, indicating that the genetic response to diversification may be lagging behind phenotypic traits (Adams et al. 2009, Avise 2009, Rheindt et al. 2011, Barth et al. 2013). Furthermore, when taken alongside the Kittlitz's Plover's extremely low intra-specific genetic structure, the observed genetic differences between the three species appear more prominent.

We predicted that the Kittlitz's Plover would be more genetically divergent from the St Helena Plover than from the Madagascar Plover given the smaller size and more isolated situation of the former population. However, there was discordance between microsatellite and DNA sequence data regarding these species' relationships. The Kittlitz's Plover appeared phylogenetically closer to

the St Helena Plover based on mitochondrial and nuclear DNA sequences but more similar to the Madagascar Plover at fast-evolving, microsatellite loci. Genetic discordance is not uncommon in phylogenetic studies and may occur due to incomplete lineage sorting, differences in selective pressures acting on different areas of the genome, or adaptive introgression (Toews & Brelsford 2012). Errors in model estimation may also lead to genetic discordance, though in this case, both sets of microsatellite analyses (STRUCTURE and PCA) were in agreement, and we evaluated sequence data directly (% sequence divergence). We therefore suggest an alternative theory. Namely, that genetic drift is likely to be stronger on the smaller, more distant island of St Helena than on Madagascar, with greater impact on the faster-evolving microsatellite loci than on the slower-evolving nuclear and mitochondrial sequences. As such, microsatellite divergence between the Kittlitz's Plover and St Helena Plover is likely to be exaggerated whilst the signals of sequence data are more likely to reflect true phylogenetic history, with the Madagascar Plover being the more distant relative of the Kittlitz's Plover and St Helena Plover.

Madagascar as a 'species pump'

Madagascar is sufficiently close to mainland Africa and large enough to offer a variety of habitats that many species have successfully colonised (Yoder & Nowak 2006). The identification of Madagascar as an important location in the genetic differentiation of the genus *Charadrius* is consistent with patterns reported for a wide range of flora and fauna, with the Mozambique Channel (approximately 400 km wide at its narrowest point) acting as a barrier to dispersal and gene flow between Madagascar and the mainland (Monaghan et al. 2005, Russell et al. 2008). In some taxa, the colonisation of Madagascar has also been reported following dispersal across the Indian Ocean, with Madagascar acting as a stepping-stone between continents. For example, trans-oceanic dispersal between Madagascar, southern Asia and Australasia (as well as mainland Africa) is thought to have played a key role in the evolutionary radiation of multiple parrot genera (Schweizer et al. 2010).

For the Charadrius plovers, genetic divergence has followed colonisation of Madagascar within each of the three African clades (Fig. 1a) and, for the Kittlitz's Plover 'superspecies' (Clade e), ancestral plovers have colonised Madagascar at least twice – the first time, resulting in the emergence of the Madagascar Plover, and the second time, leading to the presence of the modern Kittlitz's Plover population on the island. These results reflect patterns observed among other taxa, including the bat genus *Triaenops* (Russell et al. 2008) and the small minnow mayflies (Ephemeroptera: Baetidae; Monaghan et al. 2005), of multiple dispersal events from mainland Africa towards Madagascar, resulting in the emergence of several independent lineages on this island. Our results confirm the importance of Madagascar as both a biodiversity hotspot and as a location where new diversity may emerge. Thus, in the face of the current global biodiversity crisis (Woodruff 2001), our study further emphasizes the importance of conserving habitats that already foster numerous endemic species and genetically distinct populations, in the effort to preserve the future evolutionary potential of Earth's biota (Bowen 1999).

Inbreeding among island populations

That all F_{IS} values were positive and all observed heterozygosity values were lower than expected across each sampled population (and for almost all markers assessed individually) would suggest some degree of inbreeding for all populations of small plovers studied here. Furthermore, as would be predicted for island endemics with small isolated populations, we found a particularly large proportion of highly related individuals ($R > 0.5$) among the St Helena Plovers and Madagascar Plovers (65% and 42% of samples respectively, excluded from final analyses).

Nevertheless, contrary to our prediction, no strong heterozygote deficit emerged among study populations of St Helena Plovers, Madagascar Plovers, White-fronted Plovers or Kittlitz's Plovers according to microsatellite-based inbreeding coefficients (F_{IS}) regardless of whether we included or excluded highly related individuals ($R > 0.5$). Inbreeding may therefore not be a significant risk to population viability, even within the more isolated island population of the St Helena Plover.

However, with population sizes only numbering several hundred individuals, these island endemics

are nevertheless more vulnerable to extinction from adverse stochastic events or random catastrophes than are mainland plover populations that may number over several thousand, and where individuals have greater opportunity to disperse to more favourable locations (Lande 1993, Stork 2010, Warren et al. 2015).

Conclusions

The emergence of genetic differentiation between Madagascar and mainland Africa, for populations of highly mobile African Charadrius plovers, highlights the importance of islands in driving genetic divergence and ultimately allopatric speciation. This role appears most significant for high dispersers such as the Kittlitz's Plover, where mainland populations appear highly connected and genetically homogenous. Island colonisation by ancestral Kittlitz's Plovers has led to both divergence of its modern-day Madagascar population, and divergence of its two sister species, the endemic St Helena Plover and Madagascar Plover. This pattern mirrors that reported previously for the Kentish Plover, a widespread species with high genetic homogeneity across mainland Eurasia but clear genetic divergence across oceanic barriers, and multiple allopatric sister species (Küpper et al. 2012).

Despite the benefits of island colonisation for driving the formation of species, island populations often suffer costs including low population sizes and increased risk of extinction. We therefore encourage conservationists to continue working towards the protection of island populations and their increasingly threatened habitats. Careful habitat management has already begun to improve prospects for the vulnerable St Helena Plover over the last decade, offering hope of reversing the declining trends that are common for isolated island populations.

We thank Rebecca Kimball, Jon Martin Collinson and two anonymous reviewers for their valuable comments on drafts of this manuscript. For collection of samples, we thank: Marcela Beltrán, Mark Boorman, Holger Kolberg, Jorge Parra, Penn Lloyd and Wilferd Versfeld. Sequence analysis was supported by NERC-Biomolecular Analysis Facility at the University of Edinburgh and at the University of Sheffield (NBAF547, NBAF933, NBAF441). This study was funded by the NERC as

part of the PhD research of NdR. CK was supported by funding from the Max Planck Society. TS was supported by grants from the Hungarian funding agency, NKFIH (refs: K116310, KKP-126949).

Permits for fieldwork and collection of samples were provided by the national agencies in each country.

REFERENCES

- Adams, D.C., Berns, C.M., Kozak, K.H. & Wiens, J.J. 2009. Are rates of species diversification correlated with rates of morphological evolution? *Proc. R. Soc. B.* **276**: 2729-2738.
- Agnarsson, I. & Kuntner, M. 2012. The Generation of a Biodiversity Hotspot: Biogeography and Phylogeography of the Western Indian Ocean Islands. In Anamthawat-Jónsson, K. (ed.) *Current Topics in Phylogenetics and Phylogeography of Terrestrial and Aquatic Systems*. London: InTechOpen.
- Amos, W. & Harwood, J. 1998. Factors affecting levels of genetic diversity in natural populations. *Philos. Trans. R. Soc. B* **353**: 177-186.
- Avise, J.C. 2009. Phylogeography: retrospect and prospect. *Journal of Biogeography* **36**: 3-15.
- Barth, J.M.I., Matschiner, M. & Robertson, B.C. 2013. Phylogenetic position and subspecies divergence of the endangered New Zealand Dotterel (*Charadrius obscurus*). *PLoS One* **8**: e78068.
- Belliure, J., Sorci, G., Møller, A.P. & Clobert, J. 2000. Dispersal distances predict subspecies richness in birds. *J. Evol. Biol.* **13**: 480-487.
- BirdLife International & NatureServe. 2014. Bird Species Distribution Maps of the World. The IUCN Red List of Threatened Species. Version 2015.2.
- BirdLife International. 2016a. *Charadrius sanctaehelenae*. The IUCN Red List of Threatened Species 2016: e.T22693785A93423145. Downloaded on 15 January 2018.
- BirdLife International. 2016b. *Charadrius thoracicus*. The IUCN Red List of Threatened Species 2016: e.T22693780A93422870. Downloaded on 15 January 2018.
- BirdLife International. 2016c. *Charadrius pecuarius*. The IUCN Red List of Threatened Species 2016: e.T22693793A93423518. Downloaded on 15 January 2018.

BirdLife International. 2016d. *Charadrius marginatus*. The IUCN Red List of Threatened Species 2016: e.T22693828A93425312. Downloaded on 15 January 2018.

BirdLife International. 2016e. *Charadrius tricollaris*. The IUCN Red List of Threatened Species 2016: e.T22727471A94950399. Downloaded on 15 January 2018.

Blouin, M.S. 2003. DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. *Trends Ecol. Evol.* **18**: 503-511.

Bowen, B.W. 1999. Preserving genes, species, or ecosystems? Healing the fractured foundations of conservation policy. *Mol. Ecol.* **8**: S5-S10.

Cassey, P. 2002. Life history and ecology influences establishment success of introduced land birds. *Biol. J. Linn. Soc.* **76**: 465-480.

Cézilly, F., Dubois, F. & Pagel, M. 2000. Is mate fidelity related to site fidelity? A comparative analysis in Ciconiiforms. *Anim. Behav.* **59**: 1143-1152.

Chesser, R.T. 1999. Molecular systematics of the rhinocryptid genus *Pteroptochos*. *Condor* **101**: 439-446.

Claramunt, S., Derryberry, E.P., Remsen, J. V. & Brumfield, R.T. 2012. High dispersal ability inhibits speciation in a continental radiation of passerine birds. *Proc. R. Soc. B.* **279**: 1567-1574.

D'Urban Jackson, J., dos Remedios N., Maher, K.H., Zefania S., Haig, S., Oyler-McCance, S., Blomqvist, D., Burke, T., Bruford, M.W., Székely, T. & Küpper, C. 2017. Polygamy slows down population divergence in shorebirds. *Evolution* **71**: 1313-1326.

Darwin, C.R. 1845. *Journal of Researches into the geology and Natural History of the various countries visited during the voyage of H.M.S. Beagle, under the command of Captain FitzRoy, R.N.* 2nd edn. London: John Murray.

del Hoyo, J., Elliott, A. & Sargatal, J. 1996. *Handbook of the Birds of the World, vol. 3: Hoatzin to Auks*. Barcelona: Lynx Edicions.

del Hoyo, J., Collar, N.J., Christie, D.A., Elliott, A. & Fishpool, L.D.C. 2014. *HBW and BirdLife International Illustrated Checklist of the Birds of the World*. Barcelona and Cambridge: Lynx Edicions and BirdLife International.

Delany, S., Scott, D., Dodman, T. & Stroud, D. 2009. An Atlas of Wader Populations in Africa and Western Eurasia. Wageningen: Wetlands International.

dos Remedios, N., Lee, P.L.M., Burke, T., Székely, T. & Küpper, C. 2015. North or south? Phylogenetic and biogeographic origins of a globally distributed avian clade. *Mol. Phylogenet. Evol.* **89**: 151-159.

dos Remedios, N., Székely, T., Baker, N., Versfeld, W. & Küpper, C. & Lee, P.L.M. 2017. Genetic isolation in an endemic African habitat specialist. *Ibis* **159**: 792-802.

Earl, D.A. & vonHoldt, B.M. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.* **4**: 359-361.

Eberhart-Phillips, L.J., Hoffman, J.I., Brede, E.G., Zefania, S., Kamrad, M.J., Székely, T. & Bruford, M.W. 2015. Contrasting genetic diversity and population structure among three sympatric Madagascan shorebirds: parallels with rarity, endemism, and dispersal. *Ecol. Evol.* **5**: 997-1010.

Edwards, S.V., Kingan, S.B., Calkins, J.D., Balakrishnan, C.N., Jennings, W.B., Swanson, W.J. & Sorenson, M.D. 2005. Speciation in birds: genes, geography, and sexual selection. *Proc. Natl. Acad. Sci. USA* **102**: 6550-6557.

Excoffier, L. & Lischer, H.E.L. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* **10**: 564-567.

Fain, M.G., Krajewski, C. & Houde, P. 2007. Phylogeny of 'core Gruiformes' (Aves: Grues) and resolution of the Limpkin-Sungrebe problem. *Mol. Phylogenet. Evol.* **43**: 515-529.

Goudet, J. 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. *J. Hered.* **86**: 485-486.

Greenwood, P.J. & Harvey, P.H. 1982. The natal and breeding dispersal of birds. *Annu. Rev. Ecol. Systemat.* **13**: 1-21.

Groth, J.G. & Barrowclough, G.F. 1999. Basal divergences in birds and the phylogenetic utility of the nuclear RAG-1 gene. *Mol. Phylogenet. Evol.* **12**: 115-123.

Hayman, P., Marchant, J. & Prater, T. 1986. Shorebirds: an identification guide to the waders of the world. London: Croom Helm.

- Hebert, P.D.N., Stoeckle, M.Y., Zemplak, T.S. & Francis, C.M. 2004. Identification of birds through DNA barcodes. *PLOS Biology* **2**: e312.
- Hedenström, A., Klaassen, R.H.G. & Åkesson, S. 2013. Migration of the Little Ringed Plover *Charadrius dubius* breeding in South Sweden tracked by geolocators. *Bird Study* **60**: 466-474.
- Holleley, C.E. & Geerts, P.G. 2009. Multiplex Manager 1.0: a cross-platform computer program that plans and optimizes multiplex PCR. *Biotechniques* **46**: 511-517.
- Hubisz, M., Falush, D., Stephens, M. & Pritchard, J.K. 2009. Inferring weak population structure with the assistance of sample group information. *Mol. Ecol. Resour.* **9**: 1322-1332.
- Ibrahim, K.M., Nichols, R.A. & Hewitt, G.M. 1996. Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity* **77**: 282-291.
- Jakobsson, M. & Rosenberg, N.A. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **23**: 1801-1806.
- Kalinowski, S.T., Wagner, A.P. & Taper, M.L. 2006. ML-Relate: a computer program for maximum likelihood estimation of relatedness and relationship. *Mol. Ecol. Notes* **6**: 576-579.
- Kalinowski, S.T., Taper, M.L. & Marshall, T.C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* **16**: 1099-1106.
- Keenan, K., McGinnity, P., Cross, T.F., Crozier, W.W. & Prodöhl, P.A. 2013. diveRsity: An R package for the estimation of population genetics parameters and their associated errors. *Methods Ecol. Evol.* **4**: 782-788.
- Küpper, C., Edwards, S., Kosztolányi, A., AlRashidi, M., Burke, T., Herrmann, P., Argüelles-Tico, A., Amat, J.A., Amezian, M., Rocha, A., Hotker, H., Ivanov, A., Chernicko, J. & Székely, T. 2012. High gene flow on a continental scale in the polyandrous Kentish plover *Charadrius alexandrinus*. *Mol. Ecol.* **21**: 5864-5879.
- Küpper, C. & dos Remedios, N. In Press. Defining species and populations: molecular genetic studies in plovers. In Colwell, M. & Haig, S (eds) *Studies in Avian Biology*. Boca Raton: CRC Press.

Lande, R. 1993. Risks of Population Extinction from Demographic and Environmental Stochasticity and Random Catastrophes. *Am. Nat.* **142**: 911-927.

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

Linnaeus, C. 1758. *Systema naturae per regna tria naturae*. 10th ed. Rev. 2 vols. L. Salmii, Homiiae.

MacArthur, R.H. & Wilson, E.O. 1967. *The Theory of Island Biogeography*. Princeton: Princeton University Pres.

McCulloch, N.M. 2009. Recent decline of the St Helena Wirebird *Charadrius sanctaehelenae*. *Bird Conserv. Int.* **19**: 33-48.

Monaghan, M.T., Gattolliat, J., Sartori, M., Elouard, J., James, H., Derleth, P., Glaizot, O., de Moor, F. & Vogler, A.P. 2005. Trans-oceanic and endemic origins of the small minnow mayflies (Ephemeroptera, Baetidae) of Madagascar. *Proc. R. Soc. B.* **272**: 1829-1836.

Nicholls, J.A., Double, M.C., Rowell, D.M. & Magrath, D. 2000. The evolution of cooperative and pair breeding in thornbills *Acanthiza* (Pardalotidae). *J. Avian Biol.* **31**: 165-176.

Parra, J.E., Beltrán, M., Zefania, S., dos Remedios, N. & Székely, T. 2014. Experimental assessment of mating opportunities in three shorebird species. *Anim. Behav.* **90**: 83-90.

Pearson, W.J. & Colwell, M.A. 2013. Effects of nest success and mate fidelity on breeding dispersal in a population of Snowy Plovers *Charadrius nivosus*. *Bird Conserv. Int.* **24**: 342-353.

Phillimore, A.B., Freckleton, R.P., Orme, C.D.L. & Owens, I.P.F. 2006. Ecology predicts large-scale patterns of phylogenetic diversification in birds. *Am. Nat.* **168**: 220-229.

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

Prychitko, T.M. & Moore, W.S. 1997. The utility of DNA sequences of an intron from the beta-fibrinogen gene in phylogenetic analysis of woodpeckers (Aves: Picidae). *Mol. Phylogenet. Evol.* **8**: 193-204.

R Core Team. 2013. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org>.

- Reynolds, J.D. & Cooke, F. 1988. The influence of mating systems on philopatry: a test with polyandrous red-necked phalaropes. *Anim. Behav.* **36**: 1788-1795.
- Rheindt, F.E., Székely, T., Edwards, S.V., Lee, P.L., Burke, T., Kennerley, P.R., Bakewell, D.N., Alrashidi, M., Kosztolányi, A., Weston, M.A., Liu, W.T., Lei, W.P., Shigeta, Y., Javed, S., Zefania, S. & Küpper, C. 2011. Conflict between genetic and phenotypic differentiation: the evolutionary history of a 'lost and rediscovered' shorebird. *PLoS ONE* **6**: e26995.
- Richardson, D.S., Jury, F.L., Blaakmeer, K., Komdeur, J. & Burke, T. 2001. Parentage assignment and extra-group paternity in a cooperative breeder: the Seychelles warbler (*Acrocephalus sechellensis*). *Mol. Ecol.* **10**: 2263-2273.
- Rosenberg, N.A. 2004. DISTRUCT: a program for the graphical display of population structure. *Mol. Ecol. Notes* **4**: 137-138.
- Rowlands, B.W., Trueman, T., Olson, S.L., McCulloch, M.N. & Brooke, R.K. 1998. *The Birds of St Helena*. Tring: British Ornithologists' Union.
- Russell, A.L., Goodman, S.M. & Cox, M.P. 2008. Coalescent analyses support multiple mainland-to-island dispersals in the evolution of Malagasy *Triaenops* bats (Chiroptera: Hipposideridae). *J. Biogeog.* **35**: 995-1003.
- Schweizer, M., Seehausen, O., Güntert, M. & Hertwig, S.T. 2010. The evolutionary diversification of parrots supports a taxon pulse model with multiple trans-oceanic dispersal events and local radiations. *Mol. Phylogenet. Evol.* **54**: 984-994.
- Seutin, G., White, B.N. & Boag, P.T. 1991. Preservation of avian blood and tissue samples for DNA analyses. *Can. J. Zool.* **69**: 82-90.
- Shaw, M.W. 1995. Simulation of population expansion and spatial pattern when individual dispersal distributions do not decline exponentially with distance. *Proc. R. Soc. B.* **259**: 243-248.
- Slade, R.W., Moritz, C., Heideman, A. & Hale, P.T. 1993. Rapid assessment of single-copy nuclear DNA variation in diverse species. *Mol. Ecol.* **2**: 359-373.
- Stenzel, L.E., Warriner, J.C., Warriner, J.S., Wilson, K.S., Bidstrup, F.C. & Page, G.W. 1994. Long-distance breeding dispersal of snowy plovers in western North America. *J. Anim. Ecol.* **63**: 887-902.

- Stork, N.E. 2010. Re-assessing current extinction rates. *Biodivers. Conserv.* **19**: 357-371.
- Thomas, G.H., Székely, T. & Reynolds, J.D. 2007. Sexual conflict and the evolution of breeding systems in shorebirds. *Adv. Stud. Behav.* **37**: 279-342.
- Toews, D.P.L. & Brelsford, A. 2012. The biogeography of mitochondrial and nuclear discordance in animals. *Mol. Ecol.* **21**: 3907-3930.
- Warren, B.H., Simberloff, D., Ricklefs, R.E., Aguilée, R., Condamine, F.L., Gravel, D., Morlon, H., Mouquet, N., Rosindell, J., Casquet, J., Conti, E., Cornuault, J., Fernández-Palacios J.M., Hengl, T., Norder, S.J., Rijdsdijk, K.F., Sanmartín, I., Strasberg, D., Triantis, K.A., Valente, L.M., Whittaker, R.J., Gillespie, R.G., Emerson, B.C. & Thébaud, C. 2015. Islands as model systems in ecology and evolution: progress and prospects fifty years after MacArthur-Wilson. *Ecol. Lett.* **18**: 200-217.
- Wiersma, P., Kirwan, G.M. & Boesman, P. 2016a. Kittlitz's Plover (*Charadrius pecuarius*). In: del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. & de Juana, E. (eds.) *Handbook of the Birds of the World Alive*. Barcelona: Lynx Edicions (retrieved from <http://www.hbw.com/node/53830> on 17 January 2016).
- Wiersma, P., Kirwan, G.M. & Boesman, P. 2016b. White-fronted Plover (*Charadrius marginatus*). In: del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. & de Juana, E. (eds.) *Handbook of the Birds of the World Alive*. Barcelona: Lynx Edicions (retrieved from <http://www.hbw.com/node/53834> on 17 January 2016).
- Wiersma, P., Kirwan, G.M., Christie, D.A. & Boesman, P. 2016c. African Three-banded Plover (*Charadrius tricollaris*). In: del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. & de Juana, E. (eds.) *Handbook of the Birds of the World Alive*. Barcelona: Lynx Edicions (retrieved from <http://www.hbw.com/node/53832> on 17 January 2016).
- Woodruff, D.S. 2001. Declines of biomes and biotas and the future of evolution. *Proc. Nat. Acad. Sci. USA*, **98**: 5471-5476.
- Yoder, A.D. & Nowak, M.D. 2006. Has vicariance or dispersal been the predominant biogeographic force in Madagascar? Only time will tell. *Ann. Rev. Ecol. Evol. Systemat.* **37**: 405-431.

Zefania, S. & Székely, T. 2013. Charadrius spp. In Safford, R & Hawkins, F. (eds) The Birds of Africa, Volume VIII: Birds of the Malagasy Region: Madagascar, Seychelles, Comoros, Mascarenes. London: Bloomsbury Publishing.

SUPPORTING ONLINE MATERIAL

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

Table S1 GenBank Accession numbers for sequence data.

Table S2 Genetic variation and allele sizes for microsatellite loci assessed in the White-fronted Plover.

Table S3 Genetic variation and allele sizes for microsatellite loci assessed in the Kittlitz's Plover.

Table S4 Likelihoods of genetic clustering for all K values assessed using STRUCTURE.

Table S5 Sequence divergence at mitochondrial and nuclear loci.

Figure S1 Plots of genetic clustering based on STRUCTURE for three K values per dataset.

Table 1. Sampling locations and sample sizes for microsatellite analyses of plover species (genus *Charadrius*). Initial sample sizes are those prior to, and final sample sizes are those after the exclusion of samples with high relatedness ($R > 0.5$) based on microsatellite data. For sequence-based analyses, three individuals were analysed per location in all cases except for the Kittlitz's Plovers of Namibia ($n = 2$), White-fronted Plovers of South Africa ($n = 1$) and Three-banded Plovers of Kenya ($n = 1$). Madagascar sampling sites were at Andavadoaka (Anda.), Asingo and Tsimanampetsotse (Tsim.). See Figure 1 for mapped locations and species distributions.

Species	Sampling location	Initial sample size	Final sample size	% Samples excluded
Kittlitz's Plover, <i>C. pecuarius</i>	Kenya ^M	28	19	32
	Senegal ^M	14	10	29
	Anda. (Madagascar) ^I	30	21	30
	Asingo (Madagascar) ^I	11	9	18
	Tsim. (Madagascar) ^I	29	21	28
	South Africa ^{M*}	-	-	-
	All mainland	42	29	31
	All island	70	51	27
	St Helena Plover, <i>C. sanctaehelenae</i>	St Helena ^I	31	11
Tsim. (Madagascar) ^I		31	18	42
Madagascar Plover, <i>C. thoracicus</i>	(Madagascar) ^I		18	
White-fronted Plover, <i>C. marginatus</i>	Namibia ^M	20	19	5
	Anda. (Madagascar) ^I	30	19	37
	Tsim. (Madagascar) ^I	32	25	22
	South Africa ^{M*}	-	-	-
	All mainland	20	19	5
	All island	62	44	29
	Three-banded Plover, <i>C. tricollaris</i> *	Kenya ^{M*}	-	-
Tsim. (Madagascar) ^{I*}		-	-	-

^M Mainland locations; ^I island locations; * samples included in sequence-based analyses only.

Table 2. Analysis of Molecular Variance (AMOVA) for best groupings of two African plover species, the White-fronted Plover (Namibia distinct from two Madagascar sites) and Kittlitz's Plover (mainland sites in Kenya/Senegal distinct from three Madagascar sites). These groupings were those

	White-fronted Plover				Kittlitz's Plover			
	df	SS	Va	%	df	SS	Va	%
Among groups	1	131.0	2.38	46.13	1	8.8	0.08	3.73
Among populations within groups	1	5.0	0.05	1.01	3	7.6	0.01	0.53
Within populations	123	335.1	2.72	52.86	155	335.7	2.17	95.74
Total	125	471.1	5.15		159	352.1	2.26	

' variance based on separate AMOVA analyses for each possible grouping of populations.

df, degrees of freedom; SS, sum of squares; Va, variance; %, percentage of total variance.

Table 3. Pairwise F_{ST} values based on microsatellite data among populations of Kittlitz's Plovers, St Helena Plovers and Madagascar Plovers, and White-fronted Plovers, as estimated in Arlequin 3.5.2. Madagascar sampling sites were at Andavadoaka (Anda.), Asingo and Tsimanampetsotse (Tsim.).

		Kittlitz's Plover					St Helena Plover	Madagascar Plover	White-fronted Plover			
		Kenya	Senegal	Anda. (Madagascar)	Asingo (Madagascar)	Tsim. (Madagascar)	St Helena	Tsim. (Madagascar)	Namibia	Anda. (Madagascar)	Tsim. (Madagascar)	
Kittlitz's Plover	Kenya	-							White-fronted Plover	Namibia	-	
	Senegal	0.034 [‡]	-					Anda. (Madagascar)		0.432*	-	
	Anda. (Madagascar)	0.027*	0.062*	-				Tsim. (Madagascar)		0.495*	0.020 [‡]	-
	Asingo (Madagascar)	0.019	0.058 [†]	-0.012	-							
	Tsim. (Madagascar)	0.041*	0.083*	-0.006	-0.007	-						
St Helena Plover	St Helena	0.414*	0.464*	0.424*	0.512*	0.441*	-					
Madagascar Plover	Tsim. (Madagascar)	0.277*	0.322*	0.247*	0.355*	0.297*	0.692*	-				

* $p < 0.001$; [†] $p < 0.01$; [‡] $p < 0.05$

Table 4. Sequence divergence between island and mainland populations, among mainland populations, and between the Kittlitz's Plover (all populations) and its two island endemic sister species. Divergence (number of fixed differences in base pairs (bp)) between population pairs was assessed at two mitochondrial loci (COI and ND3) and four nuclear loci (ADH5, MYO2, RAG1 and FGB7) using DNAsp. Where more than two mainland sites were analysed, and for species

	Mitochondrial loci (1025 bp)			Nuclear loci (5299 bp)		
	S	Island – Mainland divergence	Mainland – Mainland divergence	S	Island – Mainland divergence	Mainland – Mainland divergence
Three-banded Plover	12	11.0 (1.1%)	-	7	2.0 (<0.1%)	-
White-fronted Plover	17	9.0 (0.9%)	4.0 (0.4%)	16	12.0 (0.2%)	0.0 (0.0%)*
Kittlitz's Plover	2	0.3 (<0.1%)	0.7 (0.1%)	51	8.0 (0.2%)	5.7 (0.1%)
Kittlitz's Plover – St Helena Plover	5	3.2 (0.3%)	-	52	6.4 (0.1%)	-
Kittlitz's Plover – Madagascar Plover	25	22.4 (2.2%)	-	58	15.0 (0.3%)	-
St Helena Plover – Madagascar Plover	22	21.0 (2.0%)	-	16	15.0 (0.3%)	-

comparisons, the mean number of fixed differences between population pairs is presented. All 'island' populations were on Madagascar, with the exception of the St Helena Plover (see Fig. 1). S, number of polymorphic sites (bp). See Table S5 for more detailed sequence divergence values.

* FGB7 sequence data not available

Table 5. Inbreeding coefficients (F_{IS}) with associated p-values based on microsatellite data, as estimated in FSTAT 2.9.3, as well as mean expected heterozygosity (H_e) and observed heterozygosity (H_o), calculated based on the equation of Nei (1987), across markers for each sampling site (see Tables S2 and S3 for details of heterozygosity per marker). Madagascar sampling sites were at Andavadoaka (Anda.), Asingo and Tsimanampetsotse (Tsim.).

Species	Sampling location	F_{IS} (p-value)	H_e	H_o
White-fronted Plovers	Namibia	0.024 (0.291)	0.449	0.438
	Anda. (Madagascar)	0.082 (0.037)	0.429	0.394
	Tsim. (Madagascar)	0.102 (0.009)	0.351	0.316
Kittlitz's Plovers	Kenya	0.120 (0.001*)	0.775	0.684
	Senegal	0.112 (0.034)	0.775	0.693
	Anda. (Madagascar)	0.202 (0.001*)	0.726	0.587
	Asingo (Madagascar)	0.086 (0.132)	0.748	0.691
	Tsim. (Madagascar)	0.070 (0.177)	0.727	0.693
St Helena Plover	St Helena	0.222 (0.112)	0.167	0.131
Madagascar Plover	Tsim. (Madagascar)	0.085 (0.128)	0.312	0.286

* Significant p-values after correction for multiple testing (adjusted nominal level for multiple comparisons: $p = 0.001$).

Figure 1. (a) An overview of phylogenetic relationships within the genus *Charadrius* (major clades CRD I and CRD II, minor clades a to f, based on dos Remedios et al. 2015). Highlighted clades (clades a, e and f) include the African study species. (b - d) Distribution maps for the three endemic African species included in population-level genetic analyses, including breeding ranges (shaded areas) and sampling locations (labelled symbols). Open circles indicate populations included in microsatellite analyses (Fig. 2); closed circles indicate populations assessed based on nuclear and mitochondrial sequence data only. An open triangle indicates the location of St Helena Plovers, analysed at microsatellite loci alongside Madagascar Plovers and Kittlitz's Plovers (all three sister species within Clade e). Madagascar Plovers were sampled at Tsimanampetsotse (Tsim.), Madagascar. Distribution maps are adapted from those provided by The IUCN Redlist of Endangered Species (BirdLife International & NatureServe 2014). Images by Wiersma et al. (2016a, 2016b, 2016c).

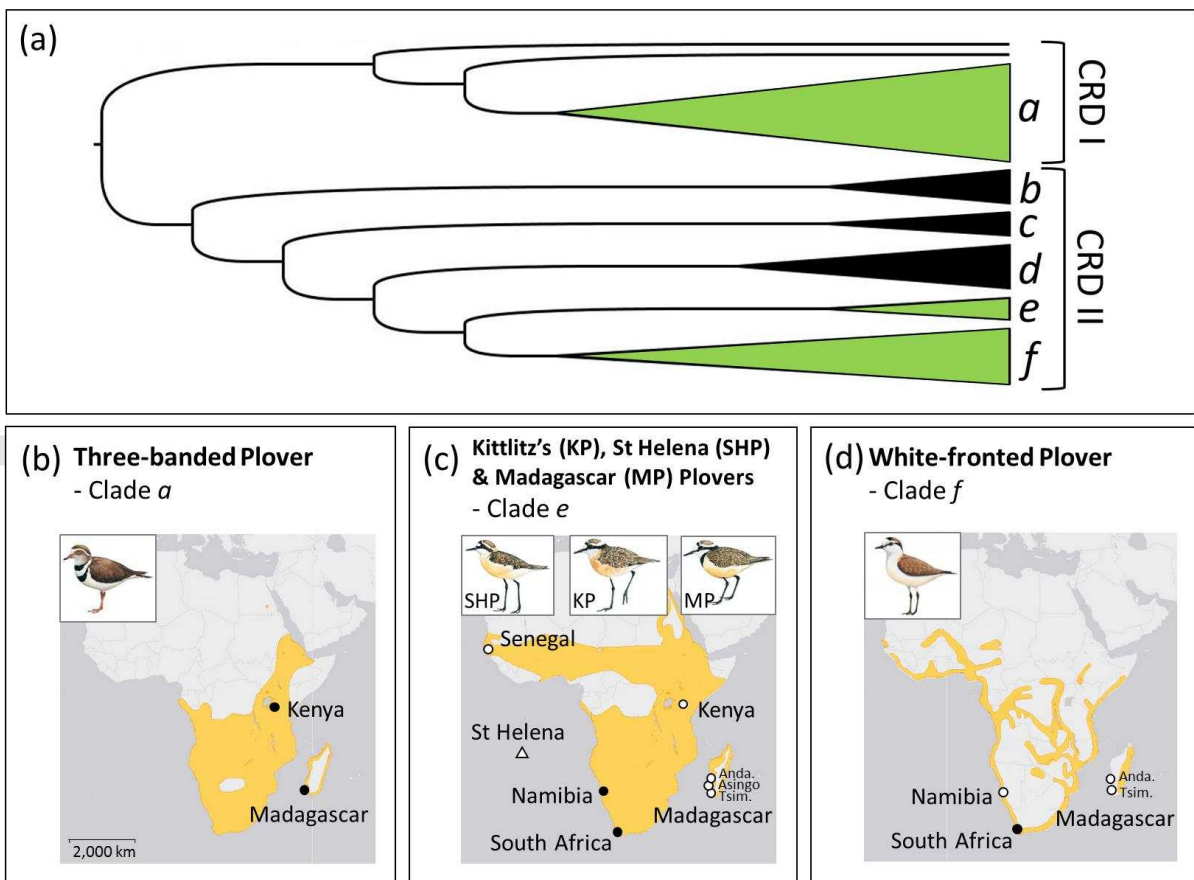
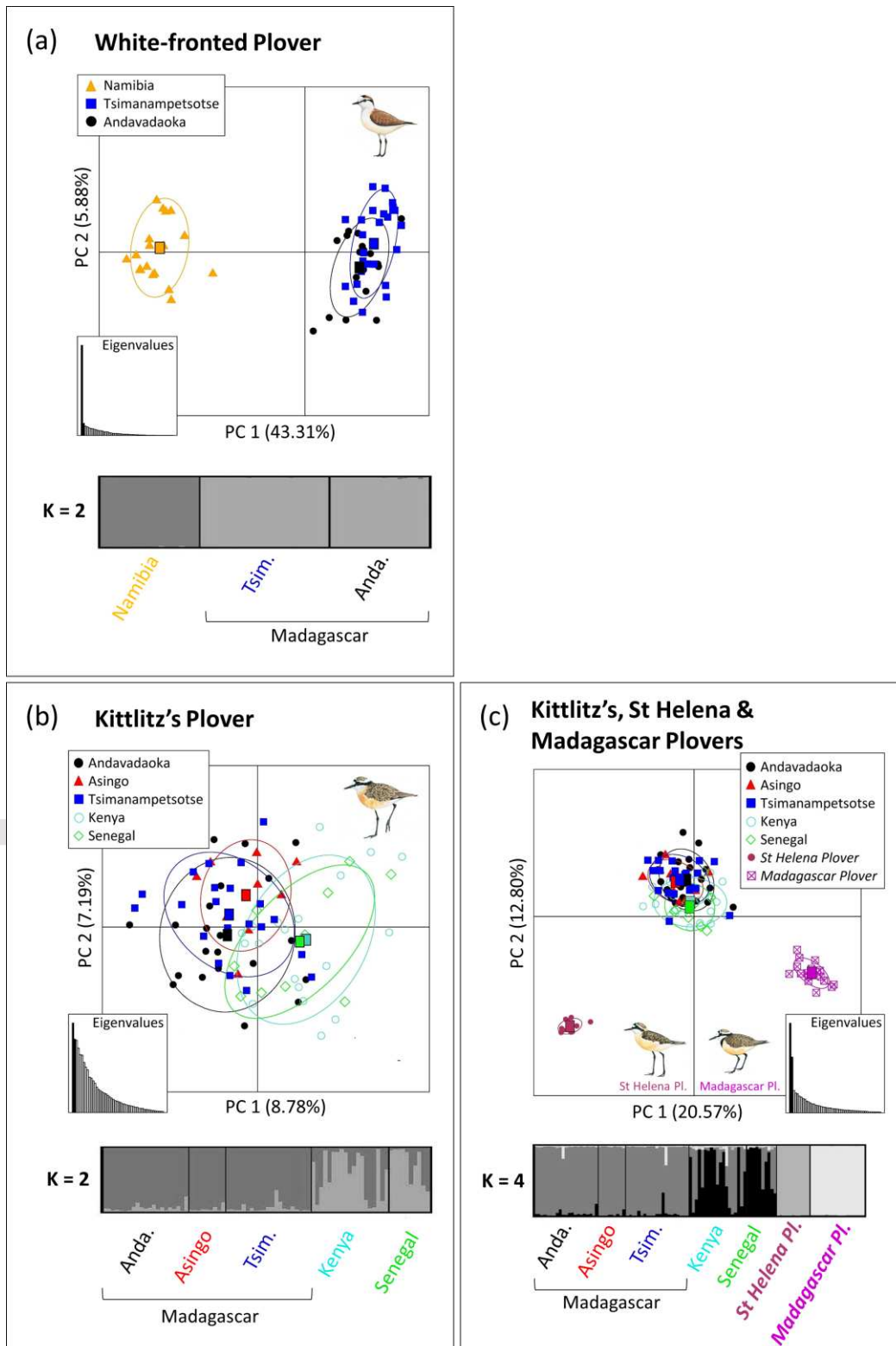
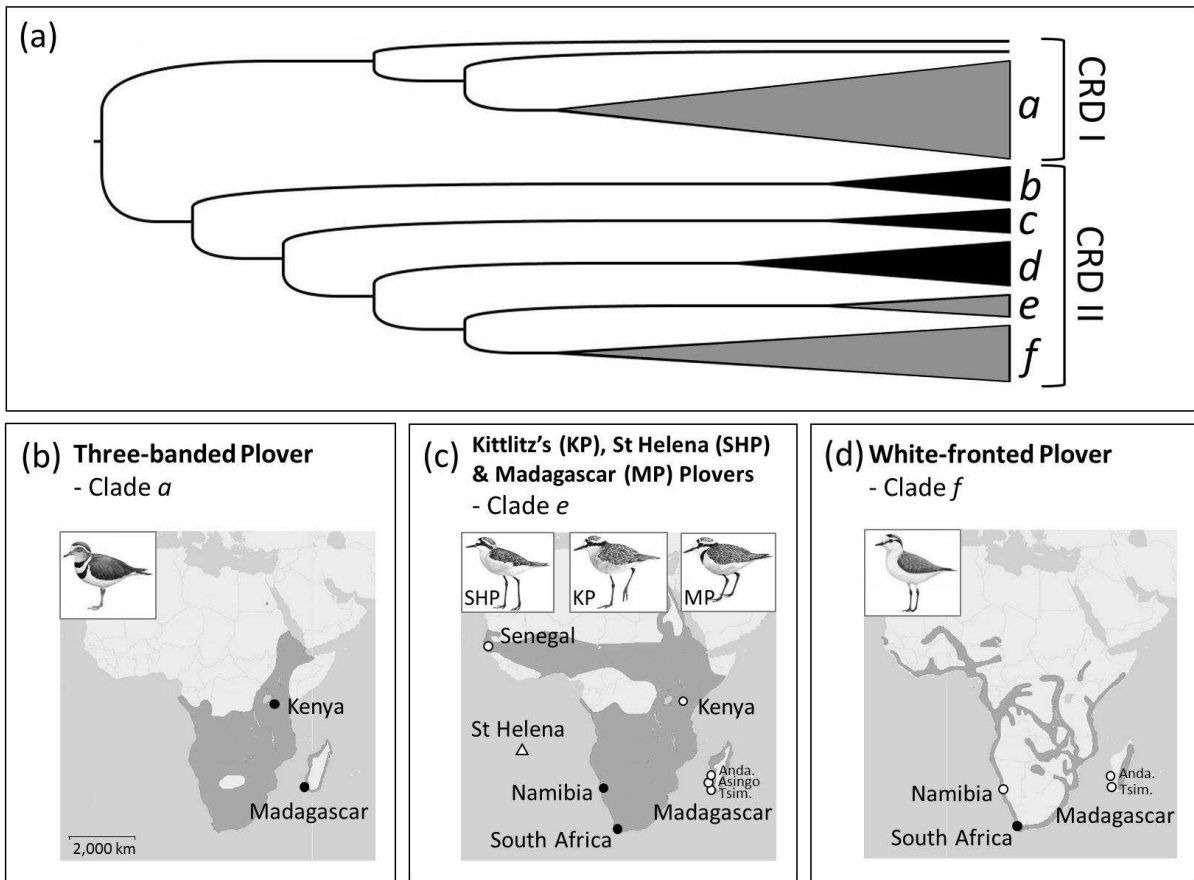


Figure 2. (a – c) Genetic clustering among plover populations based on analyses of microsatellite data. Upper charts: Principal Component Analysis (PCA); rectangles represent mean per cluster; ovals represent inertia ellipses per sampling site; eigenvalues are plotted for 50 principal components (PC 1 and PC 2 bars in black). Lower charts: STRUCTURE plots based on the number of genetic clusters (K) with highest likelihood (see Fig. S1 for plots of additional K values).



[BLACK & WHITE VERSION OF FIGURE 1 ATTACHED FOR PRINT ONLY]



[BLACK & WHITE VERSION OF FIGURE 2 ATTACHED FOR PRINT ONLY]

