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Articles

MORPHOLOGICAL AND GENETIC DIFFERENTIATION AMONG KENTISH PLOVER *CHARADRIUS ALEXANDRINUS* POPULATIONS IN MACARONESIA

DIFERENCIACIÓN GENÉTICA Y MORFOLÓGICA ENTRE LAS POBLACIONES DE CHORLITEJO PATINEGRO DE LA MACARONESIA

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SUMMARY.—Macaronesia, a group of archipelagos (Azores, Madeira, Canary Islands and Cape Verde) in the Atlantic Ocean, is one of the global biodiversity hot-spots. However, for species that occur throughout these archipelagos the extent of genetic and morphological differentiation between the archipelagos and mainland Iberia and Africa is often unknown. Here we investigate phenotypic and genetic differentiation of Kentish Plovers *Charadrius alexandrinus*, the most common breeding shorebird of Macaronesia. We show strong genetic and moderate morphological differences among breeding populations. Our results suggest that although these populations are far from being a species-

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level endemism, they deserve conservation attention given their uniqueness in terms of genetic variation. Recent loss in suitable breeding sites in Canary Islands and Azores put substantial pressure on the extant plover populations. Further studies of the conservation status and threat to these populations are needed along with a comprehensive conservation action plan to halt population decline and facilitate recovery.

Key words: Kentish Plover, phenotypic divergence, genetic structure, Macaronesia.

RESUMEN.—La Macaronesia, formada por el grupo de archipiélagos del océano Atlántico (Azores, Madeira, islas Canarias y Cabo Verde), es uno de los puntos calientes globales de biodiversidad. No obstante, para las especies que se distribuyen por estos archipiélagos se desconoce frecuentemente la magnitud de la diferenciación morfológica y genética respecto a las áreas continentales de la península Ibérica y África. Aquí investigamos la diferenciación fenotípica y genética del chorlitejo patinegro *Charadrius alexandrinus*, el ave limícola más común como nidificante en Macaronesia. Mostramos fuertes diferencias genéticas y moderadas diferencias morfológicas entre las poblaciones reproductoras. Nuestros resultados sugieren que aunque estas poblaciones están lejos de constituir un endemismo como especie, se merecen una atención de conservación dada su originalidad en términos de variación genética. La reciente pérdida de sitios de nidificación adecuados en las islas Canarias y Azores añaden presión a las poblaciones supervivientes de chorlitejos. Se necesitan más estudios sobre el estado de conservación y las amenazas a estas poblaciones junto con un plan de conservación integral para parar el declive de estas poblaciones y facilitar su recuperación.

Palabras clave: chorlitejo patinegro, divergencia fenotípica, estructura genética, Macaronesia.

INTRODUCTION

Divergence of populations may arise by limited dispersal and gradual genetic drift across populations (isolation by distance), adaptation to local environments preventing gene flow between populations (isolation by adaptation), or colonization history and founder effects (isolation by colonization, reviewed by Orsini *et al*., 2013). Given the geographic isolation that may reduce exchange of migrants, island populations are more prone to speciation than mainland populations (Adler, 1992; Emerson, 2002), and have therefore been important study systems in which to investigate fundamental ecological and evolutionary processes such as population divergence, adaptive radiation and speciation (Warren *et al*., 2015; Illera *et al*., 2016).

Macaronesia is a collection of five archipelagos located in the North Atlantic Ocean off the coast of Europe and Africa. It includes Azores, Madeira, Selvagens, Canary Islands and Cape Verde. The Macaronesian Islands are an excellent study system to investigate evolution and radiation of various taxa including plants, reptiles and birds given their substantial variation in distances from mainland and between members of the various archipelagos, and the variations in geological age of different islands (Juan *et al*., 2000; Whittaker & Fernández-Palacios, 2007; Illera *et al*., 2007; Illera *et al*., 2012). The islands of Macaronesia have a unique biogeography and given their high habitat diversity they are home to a rich endemic biota (Izquierdo *et al*., 2004; Arechavaleta *et al*., 2005; Borges *et al*., 2008, 2010), and are considered one of the global Endemic Bird Areas (Stattersfield *et al*., 1998). Much of the avifauna of the Macaronesian Islands exhibits genetic differentiation between islands or archipelagos, suggesting that there is limited gene flow between islands providing the opportunity for population differentiation

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and eventually new species to arise (Pestano *et al*., 2000; Dietzen *et al*., 2003; Kvist *et al*., 2005; Päckert *et al*., 2006; Illera *et al*., 2007). Various processes, however, threaten the unique fauna and flora of these islands. Poaching and illegal killing of marine turtles, bycatch of dolphins and sharks, logging of forests for timber and firewood, clearing vegetation for grazing and agriculture, and the introduced exotic plants and animals by humans threaten the endemic plants and animals (Garzón-Machado *et al*., 2010; Martín *et al*., 2010; Fontaine *et al*., 2011; Vasconcelos *et al*., 2013; Dutra and Koenen, 2014). As a result, many of the endemic species of the islands are seriously endangered or extinct (Hazevoet, 1995; Martín *et al*., 2010). Several studies documented that throughout the last million years island faunas have been gradually destroyed through the process of human disturbance, biological invasions and habitat exploitation (Seoane *et al*., 2011; Illera *et al*., 2012).

The Kentish Plover *Charadrius alexandrinus* is a widespread Eurasian shorebird species (Amat, 2003) that inhabits North Africa, Europe and Central and Eastern Asia (del Hoyo *et al*., 1996). A previous study of genetic differentiation between different Kentish Plover populations (Küpper *et al*., 2012) showed genetic differentiation at relatively modest distances (e.g. 40km) between mainland and island breeding sites. Here we extend this study by focusing on genetic and morphological differentiation between Macaronesian and continental Kentish Plover populations.

This study has two aims. The first is to test for morphological and genetic differentiation among Kentish Plover populations across Macaronesian region. The second is to investigate whether morphological and genetic differentiation correlates with each other as well as with geographic distance. Clarifying the morphological and genetic differentiation between these populations is warranted for two reasons. First, the breeding Kentish Plover populations in some of these archipelagos (e.g. Canary Islands) are rapidly declining (Lorenzo and Barone, 2007). Such a circumstance could prompt the extinction of differentiated populations before being considered their protection. Second, morphological and genetic differentiation between different archipelagos and mainland would imply that conservation and management of their breeding site and protection of the remaining habitats are important given the uniqueness of these populations.

METHODS

Sample collection

Morphometric measurements were collected between 1994 and 2013 during the breeding season by a total of 16 observers. Birds were caught at different localities from islands at four archipelagos (Table 1): i) Cape Verde (Boa Vista and Maio), ii) Azores (Santa Maria), iii) Canary Islands (Fuerteventura), and iv) Madeira (Porto Santo). In addition, we sampled mainland populations in two continental areas in Europe (Portugal: Samouco Salt Pans, Fuseta Salt Pans), and Africa (Morocco: Oued Gharifa Salt Pans) (Table 1, Figure 1). In Fuseta only morphological data were collected. The samples were collected using consistent methodologies. Adult plovers were caught using mist-nets or funnel traps whilst they incubated the nest or attended the chicks (Székely *et al*., 2008). All birds were ringed with uniquely numbered metal rings, and two morphological traits were measured for each adult: 1) right wing (to the nearest mm), flattened and straightened from the carpal joint to the tip of the longest primary feather; 2) right tarsus (to the nearest 0.1 mm), from the notch of the knee to the tarsus bone ends. Blood samples were collected by brachial venipunc-

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ture and stored in either Queen's Lysis Buffer (Seutin *et al*., 1991) or pure ethanol and kept at 4°C (Queen's Lysis Buffer samples) or room temperature (ethanol samples) until DNA extraction. Birds were released at their capture locations.

Morphological differences between populations

Wing length and tarsus length between sex and populations were analysed using two-way ANOVAs and Tukey HSD tests. The homogeneity of variance for the morphometric data for each sex was tested with the Bartlett's test (Snedecor and Cochran, 1989), and normality was assessed by a Shapiro-Wilk test (Shapiro and Wilk, 1965). Both tests suggested that the morphometric data had homogeneous variances and were not different from normal distribution (all $P > 0.05$). Because multiple observers measured the phenotypic traits, differences could perhaps be explained due to observer effects. To investigate this we tested for differences between archipelagos using data from two independent observers that had sampled at least two archipelagos. We found qualitatively similar differences between archipelagos (data not shown) as with using the full data set; hence we feel confident in the validity of our approach.

To assess morphological differentiation we calculated pairwise phenotypic distances (P_{ST}) between breeding locations for males and females separately. The P_{ST} index can be interpreted similarly to the commonly used F_{ST} index obtained from neutral genetic markers (Saint-Laurent *et al*., 2003; Raeymaekers *et al*., 2007). P_{ST} values close to zero indicate similarity between phenotypes whereas increasing positive values point towards high dissimilarity between phenotypes. P_{ST} values

TABLE 1

Sampling details of Kentish Plovers used in genetic and morphometric analyses.
[Detalles del muestreo de chorlitejos patinegros usados en los análisis genético y morfológico.]

Site		Population	Latitude	Longitude	Morphometric Analyses (*N*)		Genetic Analyses (*N*)
					Male	Female	
Macaronesia	Cape Verde	Boa Vista (CVB)	16°04.98'N	22°54.00'W	NA	NA	11
	Cape Verde	Maio (CVM)	15°09.00'N	23°13.02'W	301	375	25
	Azores	Santa Maria (STM)	36°58.02'N	25°09.00'W	38	38	25
	Canaries	Fuerteventura (FUV)	28°43.98'N	13°55.98'W	12	14	11
	Madeira	Porto Santo (PST)	33°04.00'N	16°21.00'W	NA	NA	2
Mainland	Portugal	Samouco (SAM)	38°44.00'N	8°59.00'W	35	35	25
	Portugal	Fuseta (FUS)	37°02.50'N	7°45.00'W	34	38	NA
	Morocco	Oued Gharifa (OUG)	35°30.00'N	6°24.00'W	10	11	25

NA no data available, *N* number of individuals. *[*NA *sin datos*. N *número de individuos.]*

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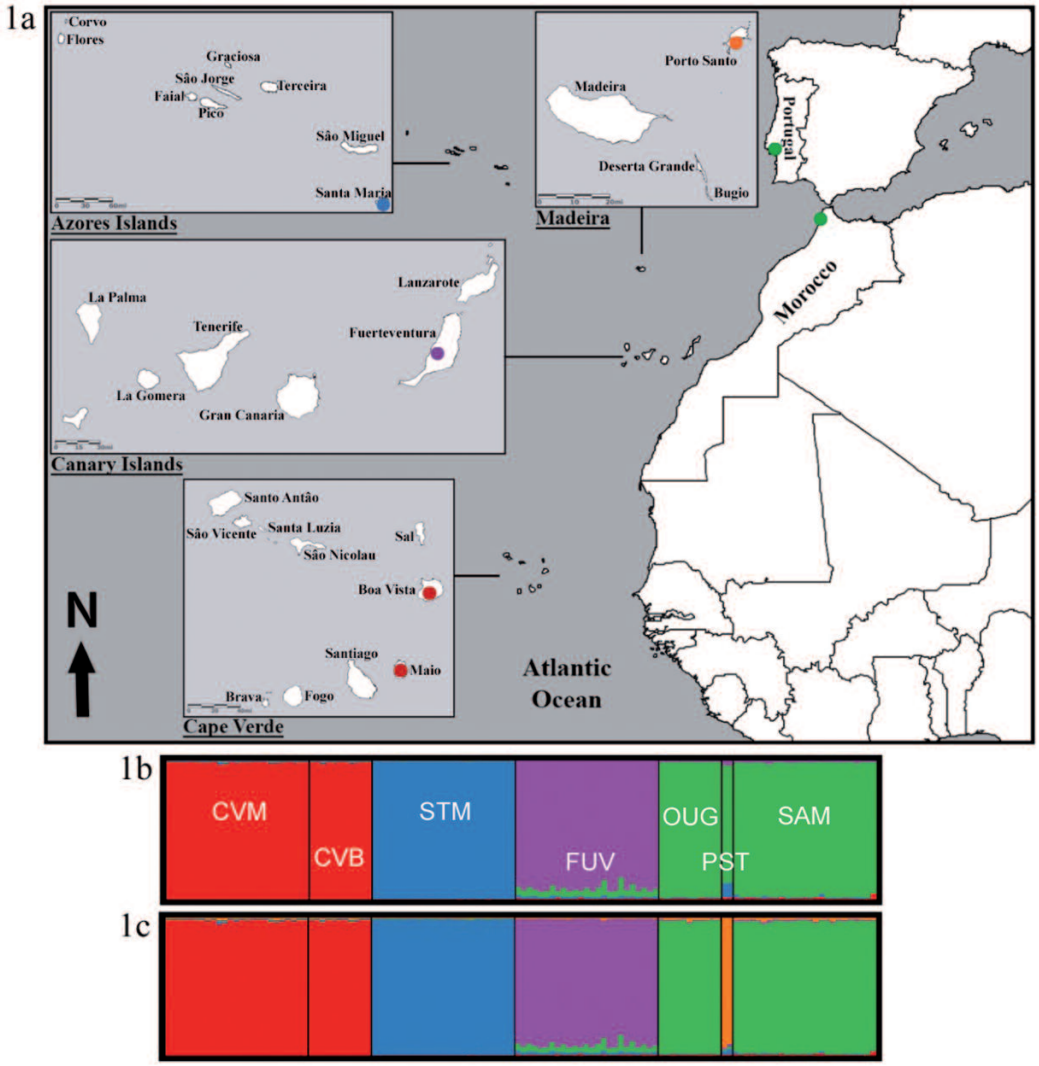


FIG. 1.—**a.** Geographic locations of seven Macaronesian Kentish Plover breeding populations. **b.** Assignment plot for Kentish Plovers from Macaronesia for best STRUCTURE model without location prior (K = 4). **c.** Assignment plot for Kentish Plovers from Macaronesia for best STRUCTURE model with location prior (K = 5). Maio (CVM), Boa Vista (CVB), Santa Maria (STM), Fuerteventura (FUV), Oued Gharifa (OUG), Porto Santo (PST), Samouco (SAM). For greyscale coding of genetic clusters see map (colour version of this figure available on the online issue).

*[**a.** Localización geográfica de siete poblaciones reproductoras de chorlitejo patinegro. **b.** Gráfico de asignación para los chorlitejos patinegros de la Macaronesia según el mejor modelo de STRUCTURE sin término de localización a priori (K = 4). **c.** Gráfico de asignación para los chorlitejos patinegros de la Macaronesia según el mejor modelo de STRUCTURE con término de localización a priori (K = 5). Maio (CVM), Boa Vista (CVB), Santa Maria (STM), Fuerteventura (FUV), Oued Gharifa (OUG), Porto Santo (PST), Samouco (SAM). Para el código de los grupos genéticos en escala de grises véase el mapa (versión en color disponible en la versión en red).]*

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of each trait were calculated separately for males and females between all population pairs using statistics derived from one-way ANOVAs, and P_{ST} was assessed as $P_{ST} = \sigma^2_{GB}/(\sigma^2_{GB} + 2\sigma^2_{GW})$ as in Sokal & Rohlf (1995), where σ^2_{GB} and σ^2_{GW} are the between- and within-population variance components of the phenotypic trait. Statistical tests and computations were conducted in R (version 3.2.2, "Fire Safety", R Core Team, 2015).

Microsatellite analyses

We re-analysed the genetic data set of Macaronesian plovers previously published (Küpper *et al*., 2012) comprising in total 124 individuals. We excluded marker C204 from the published data set because this marker amplified the same locus as marker Calex-14.

We used ARLEQUIN version 3.01 (Excoffier *et al*., 2005) to compute indices of genetic variation within and among populations including mean number of alleles (N_A), observed heterozygosity (H_O), and expected heterozygosity (H_E). Pairwise F_{ST} values among populations were used to quantify the degree of population genetic differentiation, and F_{IS} statistic to estimate the inbreeding coefficient value. The Bayesian clustering software STRUCTURE, version 2.3.4 (Pritchard *et al*., 2000), was used to determine population structure. We run two sets of models: i) without location prior as in Küpper *et al*., 2012, and ii) with location prior grouping samples according to archipelago or country. Using the location prior has been shown to identify meaningful genetic structure when the amount of available genetic data (samples or markers) is low (Hubisz *et al*., 2009). The analyses aimed to assign an individual's likelihood of belonging to a certain genetic cluster (K) based on the admixture model with correlated allele frequencies (Falush *et al*., 2003). For each approach, 15 independent simulations with K values ranging from 1 to 7 were performed for 500,000 generations with a burn-in of 50,000 generations and the five runs with the lowest Ln probability were discarded to avoid multimodality. We then assessed the assignment probabilities, logged likelihoods and, delta K (Evanno *et al*., 2005) using STRUCTURE HARVESTER (Earl & Von Holdt, 2012) to identify the most appropriate value of K. Results of the retained ten runs for each K were summarised using CLUMPP (Jakobsson & Rosenberg, 2007) and visualised with DISTRUCT (Rosenberg, 2004).

Relationships between genetic, phenotypic and geographical distances

Geographic distance was calculated between pairs of locations, evaluated using Google Earth (http://earth.google.com). To test for the relationship between genetic, phenotypic and geographic distances, we performed Mantel tests (Mantel, 1967) using matrices of pairwise F_{ST}, P_{ST}, and geographical distances (log km). Mantel tests were performed using the package ade4 in R with simulated *P* values based on 10,000 permutations (Dray and Dufour, 2007). In addition, D_{EST} (Jost, 2008) was used to evaluate genetic differentiation between populations. This metric may provide a better assessment of differentiation compared to F_{ST} (e.g. Heller & Siegismund, 2009, but see Whitlock, 2011), and we used the R package DEMEtics (Gerlach *et al*., 2010) to calculate pairwise *D* values and test whether this alternative metric of genetic distance showed a different association with geographic or morphological distances than F_{ST}. We used Bonferroni correction to calculate *P* value thresholds to account for multiple testing with the five pairwise comparisons involved (adjusted significance threshold = 0.01).

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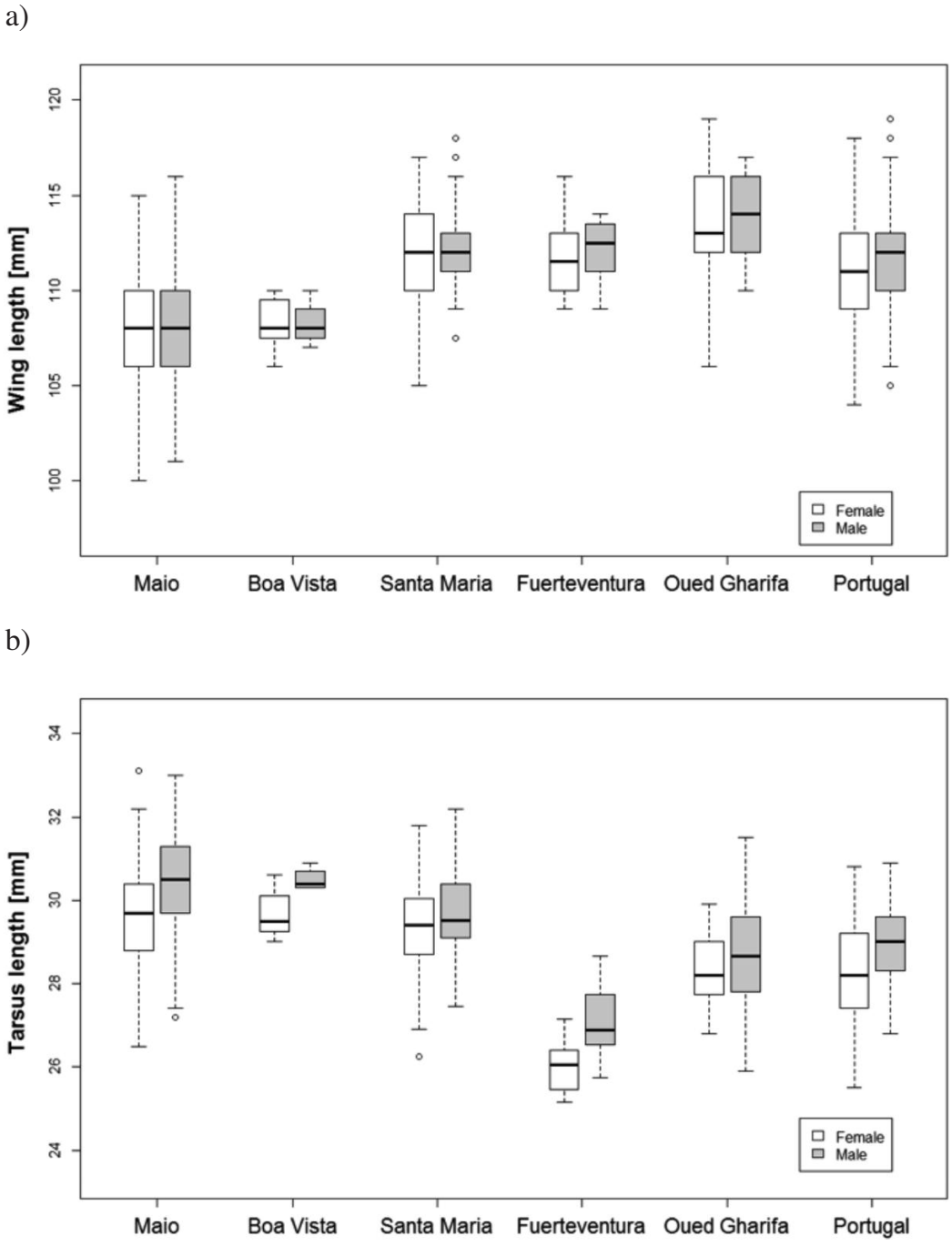


FIG. 2.—Wing length (a) and tarsus length (b) of male and female Kentish Plovers in Macaronesia. *[Longitud alar (a) y longitud del tarso (b) de los machos y las hembras de chorlitejo patinegro en Macaronesia.]*

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RESULTS

Morphological differentiation

Wing and tarsus length were significantly different between populations (Figure 2). Male plovers had longer tarsi than females (males: 30.00 ± 1.3 mm [mean ± SD], females: 29.3 ± 1.4 mm) ($F_{(1, 5)} = 87.71$, $P < 0.001$), although wing length did not differ between sexes (males: 109.5 ± 3.5 mm, females: 109.1 ± 3.4 mm) ($F_{(1, 5)} = 2.86$, $P = 0.09$). Sex differences were consistent between populations as indicated by the non-significant interaction term between sex and population (wing: $F_{(1, 5)} = 0.29$, $P = 0.92$; tarsus: $F_{(1, 5)} = 1.14$, $P = 0.34$).

Wing lengths were most similar between Fuerteventura and Santa Maria or Fuseta as indicated by the low P_{ST} values for both

TABLE 2

Pairwise morphological differentiation (P_{ST}) for male and female Kentish Plovers for a) wing length and b) tarsus length. Males are above the diagonal, females below. Significant comparisons ($P < 0.05$) are presented in bold.
[Diferenciación morfológica por pares (P_{ST}) para machos y hembras de chorlitejo patinegro en a) longitud alar y b) longitud del tarso. Los machos están sobre la diagonal, las hembras abajo. Las comparaciones significativas ($P < 0,05$) se muestran en negrita.]

a) Wing length

Population	Maio	Santa Maria	Fuerteventura	Oued Gharifa	Samouco	Fuseta
Maio		**0.36**	**0.30**	**0.49**	**0.19**	**0.34**
Santa Maria	**0.30**		–0.03	0.06	0.04	–0.01
Fuerteventura	**0.29**	–0.02		0.12	< 0.01	–0.03
Oued Gharifa	**0.46**	0.06	0.06		**0.15**	0.06
Samouco	**0.13**	0.07	0.06	**0.24**		0.03
Fuseta	**0.27**	–0.01	–0.02	0.07	**0.04**	

b) Tarsus length

Population	Maio	Santa Maria	Fuerteventura	Oued Gharifa	Samouco	Fuseta
Maio		**0.11**	**0.69**	**0.33**	**0.40**	**0.20**
Santa Maria	< 0.01		**0.58**	0.08	**0.20**	0.01
Fuerteventura	**0.71**	**0.70**		**0.31**	**0.39**	**0.64**
Oued Gharifa	**0.20**	**0.13**	**0.66**		–0.02	0.03
Samouco	**0.35**	**0.31**	**0.54**	0.03		**0.14**
Fuseta	**0.13**	**0.07**	**0.54**	–0.02	**0.08**	

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sexes (Table 2), whereas the least similar ones were between Oued Gharifa and Maio indicated by high P_{ST} values for both sexes (Table 2). Tarsus lengths were most similar between Oued Gharifa and Samouco or Fuseta, whereas the least similar ones were between Fuerteventura and Maio for both males and females (Table 2).

Genetic diversity and population differentiation

The lowest number of alleles was found in Madeira (2.31 ± 0.60, Porto Santo) whereas the highest were found in mainland Portugal (9.45 ± 3.85, Samouco, Table 3). No evidence of inbreeding was found in any of these populations as indicated by non-significant F_{IS} values (Table 3).

Pairwise F_{ST} comparisons between archipelagos (mean F_{ST} between archipelagos) showed significant genetic differentiation between archipelagos, and lower but still significant genetic differentiation between Boa Vista and Maio Kentish Plovers (i.e. within Cape Verde, Table 4). Pairwise D values demonstrated strong population structure and displayed a pattern of difference comparable to pairwise F_{ST} values for populations with $N > 2$ (Table 4).

Results from clustering analyses using STRUCTURE without location prior suggested the presence of four genetic clusters as best model splitting all archipelago populations except Madeira from the mainland population. However, when using the more sensitive method with location prior the two samples from Madeira were assigned to a separate cluster (Figure 1b). The archipelago populations were genetically distinct from the mainland population, there was only a single cluster for the two mainland populations (Iberia and North Africa), and the samples from the two Cape Verdean Islands were grouped together (Figure 1).

TABLE 3

Genetic diversity of Kentish Plovers in Macaronesia (mean ± SE). *N:* Number of individuals, N_A*:* Allele number, H_O*:* observed heterozygosity, H_E*:* expected heterozygosity.
[Diversidad genética de los chorlitejos patinegros en Macaronesia (media ± ES). N: *número de individuos,* N_A*: número de alelos,* H_O: *heterocigosidad observada,* H_E: *heterocigosidad esperada.]*

Site	*N*	N_A	H_O	H_E	F_{IS} (*P*)
Boa Vista	11	4.75 ± 1.68	0.61 ± 0.23	0.68 ± 0.12	0.10 (0.05)
Maio	25	5.80 ± 2.38	0.65 ± 0.16	0.67 ± 0.14	0.03 (0.31)
Fuerteventura	25	8.00 ± 2.75	0.76 ± 0.16	0.77 ± 0.12	0.011 (0.33)
Oued Gharifa	11	6.35 ± 2.23	0.72 ± 0.16	0.74 ± 0.14	–0.002 (0.57)
Porto Santo	2	2.31 ± 0.60	0.75 ± 0.26	0.66 ± 0.15	–0.31 (1.00)
Samouco	25	9.45 ± 3.85	0.75 ± 0.15	0.78 ± 0.12	0.03 (0.11)
Santa Maria	25	4.75 ± 1.62	0.65 ± 0.20	0.65 ± 0.17	–0.02 (0.73)

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TABLE 4

Pairwise F_{ST} values (above diagonal) and pairwise D_{EST} values (below diagonal) are shown. Significant comparisons (P < 0.05) are highlighted in bold.
[Valores de F_{ST} *por pares (sobre la diagonal) y valores de* D_{EST} *por pares (bajo la diagonal). Las comparaciones significativas* (P < 0.05) *se muestran en negrita.]*

Population	Boa Vista	Maio	Santa Maria	Fuerteventura	Porto Santo	Samouco	Oued Gharifa
Boa Vista		**0.01**	**0.18**	**0.09**	**0.25**	**0.07**	**0.11**
Maio	**0.03**		**0.18**	**0.09**	**0.24**	**0.08**	**0.11**
Santa Maria	**0.4**	**0.43**		**0.10**	**0.18**	**0.07**	**0.11**
Fuerteventura	**0.29**	**0.31**	**0.31**		**0.15**	**0.02**	**0.06**
Porto Santo	0.48	0.49	**0.33**	0.33		**0.10**	0.16
Samouco	**0.23**	**0.25**	**0.26**	**0.11**	**0.24**		**0.03**
Oued Gharifa	**0.31**	**0.31**	**0.30**	**0.23**	0.32	**0.09**	

Genetic and morphological differentiation in relation to geographic distance

The two indices of genetic differentiation F_{ST} and D_{EST} were correlated with each other (Mantel test: r = 0.93, $P < 0.001$). Genetic differentiation estimated from microsatellites tended to correlate positively with geographical distance, however, the association was not significant after correction for multiple testing (Mantel tests: r = 0.30, $P = 0.08$; r = 0.50, $P = 0.04$, for F_{ST} and D_{EST} statistics, respectively). Similarly, there was no significant association between geographic distance and morphological differentiation, or morphological and genetic differentiation (Table 5).

DISCUSSION

Our study found three major patterns. First we show that each archipelago is genetically differentiated, which is in line with previous studies using native bird populations in Macaronesia (Dietzen *et al*., 2003; Päckert *et al*., 2006; Illera *et al*., 2007; Rodrigues *et al*., 2014). Using a superior clustering algorithm that can deal better with low sample sizes (Hubisz *et al*., 2009) we also found significant and meaningful genetic differences between Madeira Kentish Plovers and the mainland population despite having only sampled two unrelated birds in this archipelago. These differences had not been detected previously (Küpper *et al*., 2012). We noted during extensive fieldwork on Cape Verde, that in contrast to mainland populations, island plovers are more monogamous and highly site faithful, and tend to return year after year to breed in the vicinity of their former territory (T. Székely pers. obs.). This limited dispersal over large number of generations may have produced the genetic difference we observed between different islands.

Second, we show that for the two morphological traits analysed there are moderate differences among populations according to ANOVA and pairwise P_{ST} comparisons. Although Kentish Plovers use fairly similar

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TABLE 5

Partial correlations between phenotypic (P_{ST}) and geographic or genetic (F_{ST}) distance matrices are shown. P value is shown in brackets.
[Correlaciones parciales entre las matrices de distancias fenotípicas (P_{ST}) y geográficas o genéticas (F_{ST}). Los valores de significación se muestran entre paréntesis.]

Variables	Males		Females	
	Wing	Tarsus	Wing	Tarsus
	r (*P*)	*r* (*P*)	*r* (*P*)	*r* (*P*)
P_{ST} and F_{ST}	0.53 (0.12)	–0.16 (0.58)	0.21 (0.33)	–0.33 (0.73)
P_{ST} and geogr. distance	0.79 (0.07)	0.06 (0.36)	0.36 (0.23)	–0.20 (0.58)

habitats across their vast geographic range that includes saltpans, sand dunes, and lake shores, it is plausible that differences in microhabitat features across Macaronesia could explain the morphological differences found. Thus, perhaps, local adaptation to different ecological settings could be driving such morphological variation between archipelagos. Alternatively, wing length and tarsus length may be involved in sexual selection: as traits used by females in mate choice decisions and/or by males competing for territories and females. Males spend substantial time on courting and displaying females (Carmona *et al*., 2015), and they also have vicious fights with other males. Body size may well influence the outcome of these fights since agility and manoeuvrability do seem to matter in mate choice decisions in shorebirds (Székely *et al*., 2004). Testing these alternatives would be highly rewarding and they will require assessing the direction and intensity of both natural and sexual selection on wing length and tarsus length.

Both indices of genetic differentiation (F_{ST} and D_{EST}) were correlated with each other. Neither of the indices of genetic differentiation was linked to morphological differentiation, nor was genetic or morphological differentiation significantly associated with geographic distance. For the former pattern, we propose that morphological differentiation is more likely driven by environmental conditions –that are idiosyncratic on each island– rather than by genetic differences. However, this needs to be investigated further by using larger sample sizes to achieve higher statistical power.

In conclusion, using a widespread shorebirds species, the Kentish Plover, as a model organism we show that Macaronesian archipelagos harbour genetically and morphologically unique populations. The detected genetic and morphological differences may warrant a review of current conservation management. We suggest that each archipelago is better treated as an independent management unit based on the detected differences in microsatellite allele frequencies between archipelagos (Moritz, 1994). Such a decision will be crucial to develop urgent specific conservation actions on those populations with an increasing level of conservation concern (e.g. Canary Islands), which populations are rapidly declining.

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REFERENCES

Adler, G.H. (1992). Endemism in birds of tropical Pacific islands. *Evolutionary Ecology*, 6: 296-306.

Amat, J.A. (2003). Chorlitejo Patinegro - *Charadrius alexandrinus*. In: Enciclopedia Virtual de los Vertebrados Españoles. L.M. Carrascal and A. Salvador (Eds.). Museo Nacional de Ciencias Naturales, Madrid. (Available electronically via http://www.vertebradosibericos.org/).

Arechavaleta, M., Zurita, N., Marrero, M.C. & Martín, J.L. (2005). *Lista preliminar de especies silvestres de Cabo Verde (hongos, plantas y animales terrestres)*. Consejería de Medio Ambiente y Ordenación Territorial, Gobierno de Canarias, 155 pp.

Borges, P.A.V., Costa, A., Cunha, R., Gabriel, R., Gonçalves, V., Martins, A.F., Melo, I., Parente, M., Raposeiro, P., Rodrigues, P., Santos, R.S., Silva, L., Vieira, P. & Vieira, V. (2010). *A List of the Terrestrial and Marine Biota from the Azores*. Princípia, Cascais, 432 pp.

Borges, P.A.V., Abreu, C., Aguiar, A.M.F., Carvalho, P., Jardim, R., Melo, I., Oliveira, P., Sérgio, C., Serrano, A.R.M. & Vieira, P. (2008). *A List of the Terrestrial Fungi, Flora and Fauna of Madeira and Selvagens Archipelagos*. Direção Regional do Ambiente da Madeira and Universidade dos Açores, Funchal and Angra do Heroísmo, 440 pp.

Carmona-Isunza, M.C., Küpper, C., Serrano-Meneses, M.A. & Székely, T. (2015). Courtship behavior differs between monogamous and polygamous plovers. *Behavioral Ecology & Sociobiology* (accepted).

Del Hoyo, J., Elliott, A. & Sargatal, J. (1996). *Handbook of the birds of the world*. Vol. 3: Hoatzin to Auks. Lynx Edicions. Barcelona.

Dietzen, C., Witt, H-H. & Wink, M. (2003). The phylogeographic differentiation of the European robin *Erithacus rubecula* on the Canary Islands revealed by mitochondrial DNA sequence data and morphometrics: evidence for a new robin taxon on Gran Canaria? *Avian Science*, 3: 115-131.

Dray, S. & Dufour, A.B. (2007). The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software*, 22: 1-20.

Dutra, A. & Koenen, F. (2014). Community-based conservation: the key to protection of marine turtles on Maio Island, Cape Verde. *Oryx*, 48: 325-329.

Earl, D.A. & von Holdt, B.M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4: 359-361.

Emerson, B.C. (2002). Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. *Molecular Ecology*, 11: 951-966.

Evanno, G., Regnaut, C. & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, 14: 2611-2620.

Excoffier, L., Laval, G. & Schneider, S. (2005). Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, 1: 47-50.

Falush, D., Stephens, M. & Pritchard, J.K. (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, 164: 1567-1587.

Fontaine, R., Giménez, O. & Bried, J. (2011). The impact of introduced predators, light-induced mortality of fledglings and poaching on the dynamics of the Cory's shearwater (Calonectris diomedea) population from the Azores, northeastern subtropical Atlantic. *Biological Conservation*, 144: 1998-2011.

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Garzón-Machado, V., González-Mancebo, J.M., Palomares-Martínez, A., Acevedo-Rodríguez, A., Fernández-Palacios, J.M., Del Arco, M.J. & Pérez-de-Paz, P.L. (2010). Strong negative effect of alien herbivores on endemic legumes of the Canary pine forest. *Biological Conservation*, 143: 2685-2694.

Gerlach, G., Jueterbock, A., Kraemer, P., Deppermann, J. & Harmand, P. (2010). Calculations of population differentiation based on G(ST) and D: forget G(ST) but not all of statistics! *Molecular Ecology*, 19: 3845-3852.

Hazevoet, C. J. (1995). *The Birds of the Cape Verde Islands*. B.O.U, Check-list No. 13. British Ornithologist's Union. Tring.

Heller, R. & Siegismund, H. R. (2009). Relationship between three measures of genetic differentiation G(ST), D(EST) and G'(ST): how wrong have we been?. *Molecular Ecology*, 18: 2080-2083.

Hubisz, M.J., Falush, D., Stephens, M. & Pritchard, J.K. (2009). Inferring weak populatio structure with the assistance of sample group information. *Molecular Ecology Resources*, 9: 1322-1332.

Illera, J.C., Emerson, B.C. & Richardson, D.S. (2007). Population history of Berthelot's pipit: colonization, gene flow and morphological divergence in Macaronesia. *Molecular Ecology*, 16: 4599-4612.

Illera, J.C., Rando, J.C., Richardson, D.S. & Emerson, B.C. (2012). Age, origins and extinctions of the avifauna of Macaronesia: a synthesis of phylogenetic and fossil information. *Quaternary Science Reviews*, 50: 14-22.

Illera, J.C., Spurgin, L.G., Rodriguez-Exposito, E., Nogales, M. & Rando, J.C. (2016). What are we learning about spéciation and extinction from the Canary Islands? *Ardeola*, 63: 15-33.

Izquierdo, I., Martín, J.L., Zurita, N. & Arechavaleta, M. 2004. *Lista de especies silvestres de Canarias (hongos, plantas y animales terrestres) 2004*. Consejería de Medio Ambiente y Ordenación Territorial, Gobierno de Canarias, 500 pp.

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.

Jost, L. (2008). G(ST) and its relatives do not measure differentiation. *Molecular Ecology*, 17: 4015-4026.

Juan, C., Emerson, B.C., Oromi, P. & Hewitt, G.M. (2000). Colonization and diversification: towards a phylogeographic synthesis for the Canary Islands. *Trends in Ecology and Evolution*, 15: 104-109.

Küpper, C., Edwards, S.V., Kosztolányi, A., Alrashidi, M., Burke, T., Herrmann, P., Argüelles-Tico, A., Amat, J.A., Amezian, M., Rocha, A., Hötker, H., Ivanov, A., Chernicko, J. & Székely, T. (2012). High gene flow on a continental scale in the polyandrous Kentish Plover *Charadrius alexandrinus*. *Molecular Ecology*, 21: 5864-5879.

Kvist, L., Kvist, J., Broggi, J.C., Illera, J.C. & Koivula, K. (2005). Colonisation and diversification of the blue tits (*Parus caeruleus* teneriffae-group) in the Canary Islands. *Molecular Phylogenetics and Evolution*, 34: 501-511.

Lorenzo, J.A. & Barone, R. (2007). Chorlitejo patinegro *Charadrius alexandrinus*. In: Lorenzo, J.A. (Ed.): Atlas de las aves nidificantes en el archipiélago canario (1997- 2003), pp. 179-182. Dirección General de Conservación de la Naturaleza-Sociedad Española de Ornitología. Madrid.

Mantel, N. (1967). The detection of disease clustering and generalized regression approach. *Cancer Research*, 27: 209-220.

Martín, J.L., Cardoso, P., Arechavaleta, M., Borges, P.A.V., Faria, B.F., Abreu, C., Aguiar, A.F., Carvalho, J.A., Costa, A.C., Cunha, R.T., Gabriel, R., Jardim, R., Lobo C., Martins, A.M.F., Oliveira, P., Rodrigues, P., Silva, L., Teixeira, D., Amorim, I.R., Fernandes, F., Homem, N., Martins, B., Martins, M. & Mendonça, E. (2010). Using taxonomically unbiased criteria to prioritize resource allocation for oceanic island species conservation. *Biodiversity and Conservation*, 19:1659-1682.

Moritz, C. (1994). Defining'evolutionarily significant units' for conservation. *Trends in Ecology and Evolution*, 9: 373-374.

Orsini, L., Vanoverbeke, J., Swillen, I., Mergeay, J. & De Meester, L. (2013). Drivers of population genetic differentiation in the wild: isolation by dispersal limitation, isolation by adaptation and isolation by colonization. *Molecular Ecology*, 22: 5983-5999.

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Pestano, J., Brown, R.P., Rodríguez, F. & Moreno, A. (2000). Mitochondrial DNA control region diversity in the endangered blue chaffinch, *Fringilla teydea*. *Molecular Ecology*, 9: 1421-5.

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

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Raeymaekers, J.A.M., Van Houdt, J.K.J., Larmuseau, M.H.D., Geldof, S. & Volckaert, F.A.M. (2007). Divergent selection as revealed by P_{ST} and QTL-based F_{ST} in three-spined stickleback (*Gasterosteus aculeatus*) populations along a coastal-inland gradient. *Molecular Ecology*, 16: 891-905.

Rodrigues, P., Lopes, R.J., Reis, S., Resendes, R., Ramos, J.A. & Cunha, R.T. (2014). Genetic diversity and morphological variation of the common chaffinch Fringilla coelebs in the Azores. *Journal of Avian Biology*, 45, 167-178.

Rosenberg, N.A. (2004). DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes*, 4: 137-138.

Saint-Laurent, R., Legault, M. & Bernatchez, L. (2003). Divergent selection maintains adaptive differentiation despite high gene flow between sympatric rainbow smelt ecotypes (*Osmerus mordax Mitchill*). *Molecular Ecology*, 12: 315-330.

Seoane, J., Carrascal, L.M. & Palomino, D. (2011). Assessing the ecological basis of conservation priority lists for bird species in an island scenario. *Journal for Nature Conservation*, 19: 103-115.

Shapiro, S.S. & Wilk, M.B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, 52: 591-611.

Snedecor, G.W. & Cochran, W.G. (1989). *Statistical Methods*. 8th ed. Iowa State University Press. Ames.

Sokal, R.R. & Rohlf, F.J. (1995). *Biometry*, 3rd edn. Freeman. New York.

Stattersfield, A.J., Crosby, M.J., Long, A.J. & Wege, D.C. (1998). Endemic Bird Areas of the World. *Bird Life Conservation Series*. Bird Life International. Cambridge, 860.

Seutin, G., White, B.N. & Boag, P.T. (1991). Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology*, 69: 82-90.

Székely, T., Freckleton, R.P. & Reynolds, J.D. (2004). Sexual selection explains Rensch's rule of size dimorphism in shorebirds. *Proceedings of The National Academy of Sciences US*, 101: 12224-12227.

Székely, T., Kosztolányi, A. & Küpper, C. (2008). Practical guide for investigating breeding ecology of Kentish Plover *Charadrius alexandrinus*. University of Bath.

Vasconcelos, R., Brito, J.C., Carranza, S. & Harris, D.J. (2013). Review of the distribution and conservation status of the terrestrial reptiles of the Cape Verde Islands. *Oryx*, 47: 77-87.

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., Rijsdijk, K.F., Sanmartín, I., Strasberg, S., 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: prospects fifty years after MacArthur-Wilson. *Ecology Letters*, 18: 200-216.

Whitlock, M.C. (2011). G'ST and D do not replace FST. *Molecular Ecology*, 20:1083-1091.

Whittaker, R.J. & Fernández-Palacios, J.M. (2007). *Island Biogeography: Ecology, Evolution, and Conservation*. Oxford University Press. Oxford.

Supplementary Electronic Material

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Table S1. Result of pairwise Tukey HSD tests for wing and tarsus length of Kentish Plovers from different Macaronesian populations.

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