Morphological and Genetic Differentiation Among Kentish Plover Charadrius alexandrinus Populations in 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-
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.

**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.*, 2012).

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 such as the White-faced Plover *Charadrius alexandrinus*, a globally uncommon species appearing to be increasing (Juan *et al.*, 2000; Whittaker & Fernández-Palacios, 2007; Illera *et al.*, 2007; Illera *et al.*, 2012).

**Key words** - Kentish Plover, phenotypic divergence, genetic structure, Macaronesia.
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, Fuzeta Salt Pans), and Africa (Morocco: Oued Gharifa Salt Pans) (Table 1, Figure 1). In Fuzeta only morphological data were collected. The samples were collected using consistent methodologies. Adult plovers were caught using mistnets 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-
tire 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

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### 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.]

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

NA no data available, $N$ number of individuals. [NA sin datos. $N$ número de individuos.]
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).]
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 $P_{ST} = \frac{\sigma^2_{GB}}{\sigma^2_{GB} + 2 \sigma^2_{GW}}$ as in Sokal & Rohlf (1995), where $\sigma^2_{GB}$ and $\sigma^2_{GW}$ 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 DISTRACT (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).
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 chorriletejo patinegro en Macaronesia.]
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 populations.

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.]

<table>
<thead>
<tr>
<th>a) Wing length</th>
<th>Population</th>
<th>Maio</th>
<th>Santa Maria</th>
<th>Fuerteventura</th>
<th>Oued Gharifa</th>
<th>Samouco</th>
<th>Fuseta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maio</td>
<td></td>
<td>0.36</td>
<td>0.30</td>
<td>0.49</td>
<td>0.19</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>Santa Maria</td>
<td>0.30</td>
<td></td>
<td>–0.03</td>
<td>0.06</td>
<td>0.04</td>
<td>–0.01</td>
<td></td>
</tr>
<tr>
<td>Fuerteventura</td>
<td>0.29</td>
<td>–0.02</td>
<td></td>
<td>0.12</td>
<td>&lt; 0.01</td>
<td>–0.03</td>
<td></td>
</tr>
<tr>
<td>Oued Gharifa</td>
<td>0.46</td>
<td>0.06</td>
<td>0.06</td>
<td></td>
<td>0.15</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Samouco</td>
<td>0.13</td>
<td>0.07</td>
<td>0.06</td>
<td>0.24</td>
<td></td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Fuseta</td>
<td>0.27</td>
<td>–0.01</td>
<td>–0.02</td>
<td>0.07</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>b) Tarsus length</th>
<th>Population</th>
<th>Maio</th>
<th>Santa Maria</th>
<th>Fuerteventura</th>
<th>Oued Gharifa</th>
<th>Samouco</th>
<th>Fuseta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maio</td>
<td></td>
<td>0.11</td>
<td>0.69</td>
<td>0.33</td>
<td>0.40</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Santa Maria</td>
<td>&lt; 0.01</td>
<td></td>
<td>0.58</td>
<td>0.08</td>
<td>0.20</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Fuerteventura</td>
<td>0.71</td>
<td>0.70</td>
<td>0.31</td>
<td>0.39</td>
<td>0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oued Gharifa</td>
<td>0.20</td>
<td>0.13</td>
<td>0.66</td>
<td>–0.02</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samouco</td>
<td>0.35</td>
<td>0.31</td>
<td>0.54</td>
<td>0.03</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fuseta</td>
<td>0.13</td>
<td>0.07</td>
<td>0.54</td>
<td>–0.02</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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.]

<table>
<thead>
<tr>
<th>Site</th>
<th>$N$</th>
<th>$N_A$</th>
<th>$H_O$</th>
<th>$H_E$</th>
<th>$F_{IS}$ ($P$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boa Vista</td>
<td>11</td>
<td>4.75 ± 1.68</td>
<td>0.61 ± 0.23</td>
<td>0.68 ± 0.12</td>
<td>0.10 (0.05)</td>
</tr>
<tr>
<td>Maio</td>
<td>25</td>
<td>5.80 ± 2.38</td>
<td>0.65 ± 0.16</td>
<td>0.67 ± 0.14</td>
<td>0.03 (0.31)</td>
</tr>
<tr>
<td>Fuerteventura</td>
<td>25</td>
<td>8.00 ± 2.75</td>
<td>0.76 ± 0.16</td>
<td>0.77 ± 0.12</td>
<td>0.011 (0.33)</td>
</tr>
<tr>
<td>Oued Gharifa</td>
<td>11</td>
<td>6.35 ± 2.23</td>
<td>0.72 ± 0.16</td>
<td>0.74 ± 0.14</td>
<td>-0.002 (0.57)</td>
</tr>
<tr>
<td>Porto Santo</td>
<td>2</td>
<td>2.31 ± 0.60</td>
<td>0.75 ± 0.26</td>
<td>0.66 ± 0.15</td>
<td>-0.31 (1.00)</td>
</tr>
<tr>
<td>Samouco</td>
<td>25</td>
<td>9.45 ± 3.85</td>
<td>0.75 ± 0.15</td>
<td>0.78 ± 0.12</td>
<td>0.03 (0.11)</td>
</tr>
<tr>
<td>Santa Maria</td>
<td>25</td>
<td>4.75 ± 1.62</td>
<td>0.65 ± 0.20</td>
<td>0.65 ± 0.17</td>
<td>-0.02 (0.73)</td>
</tr>
</tbody>
</table>
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...
habitats across their vast geographic range that includes salt pans, 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.

### 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.]

<table>
<thead>
<tr>
<th>Variables</th>
<th>Males</th>
<th></th>
<th>Females</th>
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<tbody>
<tr>
<td></td>
<td>Wing</td>
<td>Tarsus</td>
<td>Wing</td>
<td>Tarsus</td>
</tr>
<tr>
<td>$P_{ST}$ and $F_{ST}$</td>
<td>$r$ (P)</td>
<td>$r$ (P)</td>
<td>$r$ (P)</td>
<td>$r$ (P)</td>
</tr>
<tr>
<td>$P_{ST}$ and geogr. distance</td>
<td>0.53 (0.12)</td>
<td>-0.16 (0.58)</td>
<td>0.21 (0.33)</td>
<td>-0.33 (0.73)</td>
</tr>
<tr>
<td>$P_{ST}$ and geogr. distance</td>
<td>0.79 (0.07)</td>
<td>0.06 (0.36)</td>
<td>0.36 (0.23)</td>
<td>-0.20 (0.58)</td>
</tr>
</tbody>
</table>
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SUPPLEMENTARY ELECTRONIC MATERIAL

Additional supporting information may be found in the on-line version of this article. See volume 64(1) on www.ardeola.org

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

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