



High Gene Flow on a Continental Scale in the Polyandrous Kentish Plover *Charadrius alexandrinus*

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1 **High female mediated gene flow on a continental**
2 **scale in the polyandrous Kentish Plover *Charadrius***
3 ***alexandrinus***

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30

31 **Abstract**

32 **Gene flow promotes genetic homogeneity of species in time and space. Gene flow can be**
33 **modulated by sex-biased dispersal which links population genetics to mating systems.**
34 **We investigated the phylogeography of the widely distributed Kentish plover**
35 ***Charadrius alexandrinus*. This small shorebird has a large breeding range spanning**
36 **from Western Europe to Japan, and exhibits an unusually flexible mating system with**
37 **high female breeding dispersal. We analyzed genetic structure and gene flow using a**
38 **427 bp fragment of the mitochondrial (mtDNA) control region, 21 autosomal**
39 **microsatellite markers and a Z microsatellite marker in 363 unrelated individuals from**
40 **21 locations. We found no structure or isolation-by-distance over the continental range.**
41 **However, island populations had low genetic diversity, and were moderately**
42 **differentiated from mainland locations. Genetic differentiation based on autosomal**
43 **markers was positively correlated with distance between mainland and each island.**
44 **Comparisons of uniparentally and biparentally inherited markers were consistent with**
45 **female-biased gene flow. Maternally inherited mtDNA was less structured whereas the**
46 **Z-chromosomal marker was more structured than autosomal microsatellites. Adult**
47 **males were more related than females within genetic clusters. Taken together, our**
48 **results suggest a prominent role for polyandrous females in maintaining genetic**
49 **homogeneity across large geographic distances.**

50 *Keywords:* genetic diversity, genetic differentiation, microsatellites, gene flow, sex-biased
51 dispersal

52

53 *Running title:* High gene flow in plovers

54

55

56 **Introduction**

57 Investigating the link between ecology and evolution is a central challenge of population
58 biology. Dispersal has a strong influence on gene flow, genetic diversity and population
59 structure which may in turn affect the efficiency of selection and local adaptation (Bohonak
60 1999; Clobert *et al.* 2004). However, dispersal is a complex process that is often difficult to
61 assess (Edwards 1994; Okamura & Freeland 2002). For each individual, the motivation to
62 disperse often depends on age (i.e. natal or breeding dispersal), and may differ between
63 sexes. Sex-biased dispersal has been related to mating systems, resource competition and
64 inbreeding avoidance (Greenwood 1980; Lawson Handley & Perrin 2007). In socially
65 monogamous species such as many birds, local resource competition among related females
66 is predicted to lead to female-biased dispersal, whereas in polygynous species such as many
67 mammals local mate competition among related males should lead to male dispersal (Clarke
68 *et al.* 1997; Greenwood 1980; Lawson Handley & Perrin 2007). A review of mark-recapture
69 studies in birds suggested that dispersal is predominantly female-biased, although many
70 species showed no sex bias and only few studies showed male-biased dispersal (Clarke *et al.*
71 1997). However, sex-biased dispersal does not necessarily lead to sex-biased gene flow since
72 it is often not clear whether dispersers are able to successfully breed and contribute to the
73 gene pool at their new location (Prugnolle & de Meeus 2002).

74

75 The results of studies of sex-biased gene flow have challenged simplistic views on
76 associations of sex-biased dispersal with mating systems. In mammals, contrary to the
77 predictions from mating system theory, female dispersal is found in many polygynous
78 species, particularly in primates, whereas male dispersal also occurs in a number of
79 monogamous mammals (Lawson Handley & Perrin 2007). In birds, few genetic studies have
80 demonstrated female-biased gene flow (e.g. Bouzat & Johnson 2004; Johnson *et al.* 2003;
81 Piertney *et al.* 2000; Rönkä *et al.* 2008; Rönkä *et al.* 2012; Wright *et al.* 2005) and male-
82 biased gene flow is reported from a similarly small number of bird species (e.g. Capparoz *et*
83 *al.* 2009; Edwards 1994; Gibbs *et al.* 2000; Hefti-Gautschi *et al.* 2009; Liu *et al.* 2012; Mäki-
84 Petäys *et al.* 2007; Scribner *et al.* 2001). Importantly, in some birds, male-biased gene flow
85 was even found when recapture data suggested otherwise (Li & Merilä 2010).

87 Several approaches have been developed to examine sex-biased dispersal using molecular
88 markers. Studies have compared estimates for population differentiation and migration rates
89 between autosomal microsatellites and sex-specific markers (e.g. markers from non-
90 recombining chromosomal segments of the Y chromosome or mitochondrial (mt) DNA, e.g.
91 Seielstad et al. 1998; Wright et al. 2005; Lawson-Handley & Perrin 2007; Douadi et al.
92 2007). The rationale for the latter approach is that the uniparentally inherited marker is
93 shaped only by the demographic history of the sex carrying the marker. Differences in
94 estimates of population structure or gene flow between uniparentally and biparentally
95 inherited markers may therefore reveal different genetic contributions by the sexes. For
96 species in which females are dispersing and males are philopatric, genetic differentiation is
97 expected to be highest at Y-chromosomal markers, followed by autosomal markers and
98 mtDNA. However, an examination of sex-biased gene flow based only on differences
99 between biparentally and uniparentally inherited markers makes it difficult to disentangle
100 sex-biased dispersal from differences in marker characteristics such as effective population
101 sizes (which for uniparentally inherited markers is $\frac{1}{4}$ that of autosomal markers in diploid
102 monogamous systems), mutation rates or selection operating on these markers. Additionally,
103 mtDNA is often subject to bouts of natural selection, making inferences of effective
104 population size from standing levels of genetic diversity within populations challenging
105 (reviewed by Ballard & Whitlock 2004; Dowling *et al.* 2008).

106

107 To overcome these problems two alternatives have been proposed. First, sex-biased dispersal
108 may be inferred from comparisons of summary statistics between biparentally inherited
109 autosomal markers and biparentally inherited markers such as X- or Z-chromosomal markers
110 that spend more time in one sex than the other one (Carling *et al.* 2010; Li & Merilä 2010;
111 Ségurel *et al.* 2008). X (Z)-chromosomal markers undergo recombination as do autosomal
112 markers, but females (males) carry two thirds of the X (Z)-specific variation. Comparisons
113 between X (Z) markers and autosomal markers to examine sex-specific gene flow provide an
114 improvement over comparisons involving mtDNA since the differences in effective
115 population sizes are less pronounced (the effective population size of X (Z)-chromosomal
116 markers is $\frac{3}{4}$ that of autosomal markers). Second, sex-biased dispersal can be inferred by
117 comparing sex-specific summary statistics such as F_{ST} / F_{IS} -values and relatedness estimates
118 calculated for each sex separately when individuals are sampled after the dispersal event

119 (Goudet *et al.* 2002; Prugnolle & de Meeus 2002). Due to the use of a ratio, this approach
120 largely overcomes the problems caused by different effective population sizes, mutation rates
121 and selection pressures. However, this approach may only detect strong and instantaneous
122 biases because the signal is lost immediately when gene flow is followed by successful
123 reproduction. This is because the offspring will inherit randomly chosen maternal and
124 paternal alleles, thereby destroying any sex-specific pattern of differentiation built up in the
125 previous generation (Prugnolle & de Meeus 2002).

126

127 Here we investigate patterns of genetic diversity, population differentiation and sex-biased
128 gene flow in a small shorebird, the Kentish plover *Charadrius alexandrinus*. This species has
129 an unusually large geographic range including Northern Africa, Europe and Asia (Cramp &
130 Simmons 1983). Some populations breed on isolated ocean archipelagos such as Macaronesia
131 (Azores, Canary Islands, Cape Verde Islands, Madeira), and their geographical isolation may
132 reduce exchange of migrants (del Hoyo *et al.* 1996). Many Kentish plovers are polygamous
133 and have multiple clutches with one parent - usually the female - abandoning the brood to re-
134 mate whilst the remaining parent provides care for the chicks until the chicks are independent
135 (Amat *et al.* 1999; Kosztolányi *et al.* 2009; Lessells 1984; Székely *et al.* 1999; Székely &
136 Lessells 1993). The deserting female may then move large distances between different
137 breeding attempts (Székely & Lessells 1993). This female-biased breeding dispersal may
138 create high sex-biased gene flow between breeding locations.

139

140 We sampled thousands of kilometers across the breeding range of the Kentish plover,
141 including eleven mainland and ten island populations. We accomplished three things. First,
142 we compared patterns of genetic diversity between mainland and island populations, and
143 looked for signals of recent population size changes. Second, we investigated the extent of
144 genetic differentiation by including samples from breeding sites across most of its breeding
145 range. Third, we examined whether gene flow is principally driven by dispersing polyandrous
146 females during the breeding season. Because of the problems associated with the various
147 approaches to estimate sex-biased dispersal (Prugnolle & de Meeus 2002), we tested the
148 hypothesis of female mediated gene flow using three different approaches to compare genetic
149 differentiation and migration rates between mitochondrial DNA, 21 autosomal and a Z-
150 chromosomal microsatellite marker. We predicted i) lower genetic differentiation and higher

151 migration rates for mtDNA than autosomal markers, ii) stronger genetic differentiation for
152 the Z-chromosomal marker than for autosomal markers, iii) lower genetic differentiation and
153 relatedness among adult females than males.

154

155 ***Material and Methods***

156 *Sampling and molecular analyses*

157 We obtained DNA samples from 363 presumably unrelated adults or chicks of 21 Kentish
158 plover populations (20 breeding and one wintering population) in Africa and Eurasia (Table
159 1, Figure 1). Three samples of the closely related snowy plover *Charadrius nivosus* sampled
160 at Bahía de Ceuta, Mexico (23°54 N, 106°57 W) were included as an outgroup for
161 phylogenetic analyses.

162

163 To obtain DNA samples, adult plovers were trapped on the nest during incubation using
164 funnel traps (Székely *et al.* 2008) or mist nets. Chicks were caught either shortly after
165 hatching in the nest scrape or during opportunistic encounters in the field. We obtained a
166 small blood sample (25–50 µl for adults from brachial vein, 25 µl for chicks from tarsal vein)
167 for subsequent genetic analyses. Blood was stored either in Queen’s Lysis buffer (Seutin *et*
168 *al.* 1991) or absolute ethanol until extraction. All samples were collected between 1997 and
169 2009 (Table 1).

170

171 DNA extraction and amplification of 21 autosomal and one Z-linked microsatellite markers
172 followed methods described in detail in Küpper *et al.* (2007; 2009). Microsatellite genotypes
173 and sampling locations have been deposited at data dryad accession number XXXX. For
174 mtDNA analyses we used partial control region sequences described in Rheindt *et al.* (2011)
175 and amplified partial fragments of the D-loop of the control region for samples of ten
176 additional populations using the primers SNPL90 and TS778H (Funk *et al.* 2007; Wenink *et*
177 *al.* 1994) using 20-µl Polymerase Chain Reactions (PCRs). PCRs contained approximately 20
178 ng of DNA and 0.5 units of Taq DNA polymerase (Bioline) in the manufacturer’s buffer with
179 a concentration of 1.0 µM of each primer, 2.0 µM MgCl₂ and 0.20 mM of each dNTP. PCRs
180 were carried out on a thermal cycler (MJ Research model PTC DNA engine) using the

181 following program: one cycle of 3 min at 94°C followed by 35 cycles of 94°C for 30 s,
182 annealing temperature of 55°C for 30 s, 72°C for 30 s, and a final extension cycle of 10 min
183 at 72°C. To check for amplification success, we visualized 5 µl of each PCR product on a 2%
184 agarose gel stained with SYBRsafe (Invitrogen).

185

186 Products of successful PCRs were precipitated with ethanol and sequenced using Big Dye
187 Terminator Cycle chemistry on ABI 3730 capillary DNA automated sequencers at the
188 Natural Environmental Research Council Biomolecular Analysis Facility (NBAF) at the
189 University of Edinburgh. In total, a 427-bp partial sequence of the D-loop for 245 Kentish
190 plovers and three snowy plovers were available for the subsequent analysis (Table 1).
191 Sequences were aligned using the CLUSTALW algorithm implemented in CodonCode
192 Aligner 2.0.0 beta 7 and deposited in the European Molecular Biology Laboratory database
193 under accession numbers AM941516-AM941551 and HE603647-HE603792.

194

195 *Statistical analyses*

196 We used ARLEQUIN version 3.1 (Excoffier *et al.* 2005) and DNASP version 5 (Librado &
197 Rozas 2009) to calculate the following mtDNA indices of genetic diversity for each sampling
198 location: number of haplotypes n_{HT} , haplotype diversity h and nucleotide diversity π . For
199 autosomal microsatellites we calculated observed (H_o) and expected heterozygosity (H_e) in
200 ARLEQUIN and allelic richness A_{rich} using the ‘StandArich’ package in R (available from
201 <http://www.ccmr.ualg.pt/maree/software.php?soft=sarich>). A_{rich} was adjusted to the minimal
202 sample per location among the breeding populations (PST: $n = 2$, Table 1). Note that the
203 results did not qualitatively change if we exclude locations where few individuals were
204 sampled (i.e. less than 5 individuals, results not shown). We then compared genetic diversity
205 indices that take into account sample size (π , A_{rich} , H_o) between island and mainland
206 breeding locations using Wilcoxon rank sum tests.

207

208 We tested for genetic bottlenecks and demographic changes in two ways. First, for mtDNA
209 we calculated Tajima’s D using the program DNASP (Librado & Rozas 2009). Negative
210 Tajima’s D values, if the marker is deemed neutral, may suggest a population expansion after

211 a bottleneck whereas positive values may suggest population size decrease. Second, for
212 autosomal microsatellites we used the coalescent method implemented in the program
213 BOTTLENECK (Cornuet & Luikart 1996) and tested whether observed heterozygosity
214 excess or deficiencies were indicative of a recent bottleneck or population expansion which
215 would follow a bottleneck after colonization. As model for microsatellite evolution we chose
216 the two phased model (TPM) and tested for statistical significance with Wilcoxon signed-
217 rank tests.

218

219 To test for association of geography with mtDNA we carried out Bayesian phylogenetic
220 analyses. The most appropriate model of sequence evolution was selected in
221 MRMODELTEST 2.2 based on Akaike's information criterion (Akaike 1974; Nylander
222 2004). The Bayesian analysis was conducted using MRBAYES 3.1 (Ronquist & Huelsenbeck
223 2003). We conducted three analyses with different *a priori* topologies: (1) without constraints
224 of the sample origin ('Unconstrained'), (2) constraining samples from island populations to a
225 monophyletic origin ('Islands Constrained') and, (3) constraining all samples of the same
226 location to monophyletic origins ('All Constrained'). For each topology we conducted the
227 Bayesian analyses using four Markov chains at four different temperatures. Markov chains
228 were sampled every 3000 generations and run for 30 million generations. After completion
229 we checked for chain convergence and removed a burn-in of 25% (7.5 million generations).
230 The most likely topology was chosen based on Bayes factors (Kass & Raftery 1995;
231 Nylander *et al.* 2004).

232

233 Genetic differentiation among populations was estimated in three ways. First we calculated
234 Φ_{ST} -values (mtDNA), F_{ST} - and R_{ST} -values (microsatellites) in ARLEQUIN. R_{ST} is expected
235 to give more accurate differentiation estimates than traditional F_{ST} if the mutation process of
236 the genetic markers resembles a stepwise process (Balloux & Lugon-Moulin 2002; Slatkin
237 1995). Pairwise differentiation coefficients were calculated between all 21 locations.
238 Permutation tests with 1000 randomly generated $\Phi_{ST} / F_{ST} / R_{ST}$ -values were used to test the
239 probability of observed values arising by chance. Significance levels were adjusted using q-
240 values to account for false discovery rates due to multiple testing (Storey 2002). Second, we
241 used factorial correspondence analysis (FCA) to examine genetic differentiation of

242 multilocus genotypes using the program GENETIX version 4.05 (Belkhir *et al.* 1996-2004).
243 FCA is a multidimensional statistical method to visualize data that is superior to principal
244 component analysis when discrete variables such as co-dominant microsatellite loci are
245 involved. Third, we used two Bayesian clustering approaches to examine population
246 differentiation with the autosomal markers. We used STRUCTURE version 2.1 (Pritchard *et*
247 *al.* 2000) to estimate the number of clusters K in our data set and to assign individuals based
248 on the admixture model with correlated allele frequencies to one or several clusters. With this
249 approach a proportion of each individual's genome is assigned to each cluster assuming gene
250 flow among populations. We ran ten independent simulations with 500,000 generations
251 following a burn-in of 250,000 for K ranging from 1 (no differentiation) to 21 (maximum
252 divergence). We evaluated the assignment probabilities, log likelihood and ΔK (Evanno *et al.*
253 2005) to determine the optimal number of clusters. We then used the program TESS version
254 2.3.1 (Chen *et al.* 2007) to assign individuals to clusters and validate the number of clusters
255 estimated with STRUCTURE. In TESS we used the hierarchical mixture model where the
256 prior distribution on cluster labels is determined by a Hidden Gaussian Random Field (CAR
257 model). This approach may provide lower error rates than other clustering methods when
258 low levels of genetic structure are observed (Chen *et al.* 2007). For each K we ran 50
259 iterations with 50,000 cycles after discarding a burn-in of 30,000 cycles and chose the best 10
260 runs (20%) according to the lowest Deviance Information Criterion (DIC) values. Average
261 DIC values for each K were plotted and the most likely K was determined at the value where
262 DIC values reached a plateau. For both STRUCTURE and TESS we averaged the results of
263 the best ten runs using CLUMPP (Jakobsson & Rosenberg 2007). Results of the processed
264 runs were visualized with DISTRUCT version 1.1 (Rosenberg 2004).

265

266 We tested for isolation-by-distance in two ways. First, we used the Mantel's test implemented
267 in ARLEQUIN to test for a general association of geographic distance with genetic
268 differentiation using all sampled breeding population locations. Second, we carried out a
269 linear regression to test whether the genetic differentiation of island populations was affected
270 by their log-transformed distance to the mainland. We used the largest distance of open water
271 that plovers originating from the mainland needed to cross in order to reach the island
272 breeding locations ('ocean distance'), because we reasoned that plovers would use islands
273 between the mainland and island breeding locations as stepping stones. As measure of

274 genetic differentiation we used Rousset's distance ($F_{ST} / (1-F_{ST})$ for microsatellites or $\Phi_{ST} /$
275 $(1-\Phi_{ST})$ for mtDNA, (Rousset 1997). Genetic differentiation was calculated for pairwise
276 comparisons with each island population versus the entire mainland population and we
277 estimated distances with the ruler function in Google Earth version 4.02 (Google 2007).
278 Distances were \log_{10} transformed before the analyses.

279

280 To estimate number of migrants ($4N_e m$ for microsatellites and $2N_e m$ for mtDNA) and the
281 Watterson estimators Θ we used the coalescent approach implemented in MIGRATE version
282 3.2.6. We estimated $4N_e m / 2N_e m$ between and Θ within all population clusters previously
283 identified through STRUCTURE and TESS. After an initial burn-in of 25,000,000 / 100,000
284 (mtDNA / autosomal microsatellites) a long chain of 50,000,000 / 1,000,000 trees were
285 sampled of which 50,000 / 2,000 trees were recorded. Four-chain heating was used with
286 temperatures set to 1, 1.2, 3, and 6 to improve tree space sampling. Each run was replicated
287 five times and the Bayesian estimates of the previous run were used as initial estimates of
288 these parameters for the subsequent run and the values of the last chain was recorded. Two
289 independent runs were carried out to confirm that final chains converged at highly similar
290 estimates for modes and 95% confidence intervals and we report the mean values of the two
291 analyses.

292

293 We tested for sex-biased dispersal by comparing genetic estimates of migration and genetic
294 differentiation of biparentally and uniparentally inherited markers in three ways. First,
295 following Wright *et al.* (2005) we compared migration rates of mtDNA and autosomal
296 microsatellites calculated in MIGRATE. The effective population size (N_e) of maternally
297 inherited mtDNA is only one fourth of biparentally inherited nuclear markers (Avisé 2004).
298 If the adult sex ratio of a population is 1:1, $4N_e m$ estimates of nuclear markers divided by
299 four can be compared with $2N_e m$ rates of mtDNA. Differences are attributed to sex-biased
300 dispersal. Second, we compared pairwise F_{ST} values derived from the Z-linked microsatellite
301 marker with the values of the 21 autosomal microsatellites for all locations where we
302 sampled at least two males ($n = 20$, PST was excluded). We only included genotypes of
303 males for the calculation of the coefficient of genetic differentiation derived from the Z-
304 linked marker, since females have only a single copy of the Z chromosome. We used a

305 Wilcoxon signed-rank test to examine statistical significance of autosomal and Z-
306 chromosomal F_{ST} differences. No difference in genetic differentiation between Z and
307 autosomal markers would suggest lack of sex-biased gene flow. Stronger differentiation at
308 the Z marker than the autosomal markers indicates female-biased gene flow whereas lower
309 differentiation suggests male-biased gene flow. Third, we used a randomization method
310 implemented in FSTAT to test whether pairwise F_{ST} , F_{IS} and relatedness differ between sexes
311 (Goudet *et al.* 2002). The rationale for this test is that genetic differentiation and relatedness
312 will differ between sexes if one sex largely stays at the natal site whereas the other sex
313 disperses. For this approach the difference between the genetic indices of differentiation or
314 relatedness of adults from both sexes are calculated and then the sex is randomly assigned to
315 each multilocus genotype of the original population sample keeping the original sex ratio
316 intact. Since the analysis is sensitive to sample sizes and power decreases with small sample
317 sizes we used the clusters previously identified with the Bayesian analyses to define
318 populations and repeated this procedure 1000 times to examine statistical significance
319 (Goudet *et al.* 2002).

320

321 Statistical analyses were conducted in R 2.10.1 (R Development Core Team 2010). Analyses
322 involving the software STRUCTURE, TESS and MIGRATE were carried out on the Odyssey
323 Computing Cluster at Faculty of Arts and Science, Harvard University.

324

325 ***Results***

326 *Genetic diversity and tests for bottlenecks*

327 The 427 bp fragment of the Kentish plover control region contained 34 (8.0%) polymorphic
328 sites. Among 237 plovers sampled at breeding locations we found 51 haplotypes (54
329 haplotypes among 245 samples from all 21 locations). Thirty-three haplotypes were exclusive
330 to plovers from mainland breeding locations, ten haplotypes were exclusively found in
331 plovers from island breeding sites and only eight haplotypes were shared between island and
332 mainland breeding locations. However, the shared haplotypes included the three most
333 frequently observed haplotypes and accounted for more than 50% of the haplotypes observed
334 in both groups (Islands: 56.5%, Mainland: 63.2%).

335

336 Genetic diversity measured by microsatellite markers was significantly higher for mainland
337 than island populations (Figure 2, Table 2, A_{rich} : Wilcoxon-rank-sum-test: $W = 94$, $P < 0.001$,
338 $n = 20$; H_o : Wilcoxon-rank-sum-test: $W = 76$, $P = 0.047$, $n = 20$). However, there was no
339 significant difference in genetic diversity between mainland and island breeding locations
340 based on mtDNA (π : Wilcoxon-rank-sum-test: $W = 68$, $P = 0.17$, $n = 20$).

341

342 We did not find evidence for population expansion, population reduction or selection in
343 mtDNA. Tajima's D values based on mitochondrial haplotypes and coalescent analyses based
344 on the microsatellites were nonsignificant for all mainland or island sites (Table 2).
345 However, two Atlantic island populations had significant heterozygosity excess in
346 microsatellites, suggesting recent population decline (Wilcoxon signed rank test: STM: $P =$
347 0.0001 ; FUV: $P = 0.007$). The coalescent analysis based on the microsatellite genotypes also
348 revealed a mode shift of the allele frequency distribution for the STM but not the FUV
349 population under the TPM model providing further support for the recent population
350 reduction hypothesis at STM.

351

352 *Phylogenetic analyses*

353 Bayesian phylogenetic analyses of the mitochondrial data were carried out with the
354 $GTR+I+G$ model of sequence evolution. The 'Unconstrained' model received the highest
355 support, followed by 'Islands Constrained' ($2\log_e(B10) = 9.46$) and lastly 'All Constrained'
356 ($2\log_e(B10) = 11.3$). The 'Unconstrained' model had little association with geography, since
357 Kentish plovers from island and mainland populations were grouped together, branch lengths
358 were short, or support was ≤ 0.95 (Figure S1). Only five nodes were supported by ≥ 0.95 , and
359 their branches contained samples from one island population (TWB), and four mainland
360 populations from the center of the Kentish plover distribution (Figure S1: ELT; ALW; ALW
361 & XIN; KUJ).

362

363 *Genetic differentiation*

364 Pairwise comparisons for mainland-mainland breeding sites revealed very low (or complete
365 lack of) genetic differentiation across autosomal and sex-specific markers. R_{ST} values for
366 microsatellite data and the majority of F_{ST} and Φ_{ST} values were low and nonsignificant for
367 mainland-mainland comparisons (Tables S1 and S2). Only mitochondrial Φ_{ST} values between
368 one of the locations from the center of the continental distribution (ALW) and the three
369 Iberian locations (SAM, FDP, DON) were significant but the Φ_{ST} values were low and we
370 interpret these results rather as stochastic effects of a single marker than biologically
371 meaningful. None of the F_{ST} values calculated from the Z-linked marker were significant but
372 13 of the 55 pairwise F_{ST} values for autosomal markers for mainland comparisons were.
373 However, no autosomal F_{ST} value was larger than 0.03. Φ_{ST} values ranged from -0.05 to 0.14
374 (mean = 0.02, SE = 0.008), F_{ST} ranged from -0.01 to 0.03 (mean = 0.01, SE = 0.002) and R_{ST}
375 ranged from -0.02 to 0.04 (mean = 0.01, SE = 0.002) for mainland sites for which at least 10
376 individuals were sampled. For breeding sites that were separated by open ocean and for
377 which at least ten individuals were sampled most Φ_{ST} , F_{ST} and R_{ST} comparisons were highly
378 significant. Φ_{ST} values ranged from -0.01 to 0.58 (mean = 0.22, SE = 0.019), F_{ST} values
379 ranged from 0.02 to 0.17 (mean = 0.07, SE = 0.001) and R_{ST} ranged from 0 to 0.22 (mean =
380 0.08, SE = 0.006).

381

382 Genetic differentiation did not follow an isolation-by-distance model, neither for the full data
383 set (Mantel tests for autosomal microsatellites: $B = 0.000004$, $P = 0.11$; Z microsatellite: $B =$
384 0.000005 , $P = 0.19$; mtDNA: $B = 0.000012$, $P = 0.064$), nor for the partial data set that
385 included only the mainland locations (Mantel tests for autosomal microsatellites: $P = 0.99$; Z
386 microsatellite: $P = 0.73$; mtDNA: $P = 0.13$). The FCA analysis corroborated the lack of
387 genetic differentiation among mainland sites (Figure 3) although only 3.4% of the genetic
388 variation was described by the two first axes. Multilocus genotypes of plovers from distant
389 geographic locations in Eurasia and Africa clustered together. Similarly, samples from PST,
390 FAR and FUV were only poorly differentiated from the continental cluster whereas most
391 samples from the Cape Verde Archipelago (CVB & CVM), East Asian Islands (TWB, OKN,
392 JAP) and STM were aggregated into separate clusters.

393

394 Genetic differentiation between island and mainland locations for autosomal microsatellites
395 (but not mtDNA or the Z-linked marker) was predicted by ocean distance between island
396 breeding locations and the mainland (Figure 4, autosomal microsatellites: $B = 0.04$, $r^2 = 0.56$,
397 $df = 7$, $P = 0.02$; Z microsatellite: $B = 0.15$, $r^2 = 0.23$, $df = 7$, $P = 0.19$; mtDNA: $B = 0.17$, r^2
398 $= 0.08$, $df = 7$, $P = 0.46$).

399

400 The two Bayesian analyses for cluster assignment suggested $K = 5$ as the most likely number
401 of population clusters. Both analyses consistently flagged three separate clusters (Figure 1):
402 the Azores (STM), Cape Verde (CVB & CVM) and East Asian Islands (TWB, OKN & JAP).
403 The wintering population (TWW) was intermediate between the Eastern Asian cluster and
404 continental Eurasian Kentish plovers; a number of individuals had largely continental
405 genotypes suggesting that Kentish plovers from the mainland overwinter in Taiwan. There
406 was disagreement about assignment to the remaining clusters between TESS and
407 STRUCTURE. Results of STRUCTURE suggested two additional clusters: one for breeders
408 from the Canary Islands (FUV) and one for the breeders from Farasan Islands (FAR) with the
409 genomes of mainland Kentish plovers split about equally between these two clusters (Figure
410 1). The genotypes of the two samples from PST were split between the East Asian and the
411 FUV cluster. Results of TESS suggested one cluster for the mainland Kentish plovers, and
412 assigned the majority of the genotypes of the FUV, PST and FAR plovers to this cluster.
413 However, a significant portion of the genomes (0.19 and 0.12, respectively) of the FUV and
414 FAR plovers were attributed to a joint fifth cluster. For FUV and FAR plovers other
415 significant portions of the genomes were assigned to the CVB/CVM and STM clusters. In
416 TESS runs with higher K values assigned these parts of the FUV and FAR plover genomes to
417 different clusters although the largest part of their genomes was still assigned to the mainland
418 cluster.

419

420 *Migration*

421 Because of the uncertain assignment of FAR and FUV breeders, we calculated migration
422 rates assuming six genetic clusters: 1) STM, 2) CVB & CVM, 3) FUV, 4) Mainland, 5) FAR
423 and 6) TWB, OKN & JAP. The two samples of breeders from PST were excluded from the

424 migration analysis. Results of the two independent runs were consistent and very similar
425 indicating that the runs had converged.

426

427 The results of the coalescent analysis showed that island population clusters exchanged few
428 migrants (Table 3). However, island population exchanged migrants with the mainland
429 cluster. MtDNA and microsatellites suggested unequal gene flow with more plovers tending
430 to migrate from islands to the mainland than from mainland to islands.

431

432 *Sex-biased dispersal*

433 Comparisons using biparentally and uniparentally inherited markers supported the hypothesis
434 of moderately female-biased gene flow. After adjusting for different N_e (by dividing nuclear
435 estimates by four under the assumption of equal sex ratios) modal values for total migration
436 rates (immigration and emigration rates combined) were higher for mtDNA than for
437 microsatellites (Table 3). Modal values for Nm from the islands to the mainland were on
438 average two to four times higher for mtDNA, than modal values estimated from
439 microsatellite markers although the Nm estimates from mtDNA showed large confidence
440 limits. By contrast, Nm estimates were lower for mtDNA markers than microsatellite markers
441 for gene flow from mainland to island clusters across all comparisons.

442

443 The Z-chromosomal microsatellite marker exhibited more genetic structure than the 21
444 autosomal markers (Median $F_{ST\ Z\delta} = 0.042$; Median $F_{ST\ aut} = 0.036$, $P = 0.003$). Genetic
445 differentiation tended to be higher in adult males than females for two of the three tests that
446 compared summary statistics of biparentally inherited markers between the sexes ($F_{ST\delta} =$
447 0.063 , $F_{ST\varphi} = 0.049$, $P = 0.068$; $R_{\delta} = 0.12$, $R_{\varphi} = 0.09$, $P = 0.058$), although there was no such
448 trend in F_{IS} ($F_{IS\delta} = 0.022$, $F_{IS\varphi} = 0.037$, $P = 0.24$).

449

450 **Discussion**

451 Our results demonstrate unusually high gene flow across large geographic distances in a
452 terrestrial bird species using mtDNA, autosomal and Z-linked microsatellites. Bayesian

453 analyses of mtDNA and autosomal microsatellite loci show that mainland Kentish plovers are
454 largely genetically undifferentiated across continental Eurasia and Africa. The genetic pattern
455 of continental sampling locations which were separated by up to 10,000 km resembled the
456 pattern in a single panmictic population. This lack of genetic structure cannot be explained by
457 homoplasy of microsatellite markers or, by low power of the applied marker set, since we
458 detected genetic differentiation of ocean island populations and the panmixia pattern derived
459 from mtDNA was consistent with the pattern observed at microsatellites. Island populations
460 were moderately differentiated from the mainland populations and genetic differentiation
461 increased with distance of the islands from mainland.

462

463 When analyzing patterns of genetic differentiation it is important to disentangle current gene
464 flow from demographic processes that occurred in the population history (Avice 1994). Low
465 genetic structure and sharing of haplotypes are seen in many species that have undergone a
466 bottleneck and shifted their geographic distributions in response to climate oscillations such
467 as the last glacial maximum (e.g. Hewitt 2000; Wenink *et al.* 1994). However, we argue that
468 it is unlikely that the last glacial maximum has caused a profound shift of the Kentish plover
469 distribution. First, in contrast to inhabitants of higher latitudes most of the present distribution
470 of Kentish plovers was not covered by the ice sheet during the last glacial maximum. The
471 center of the current distribution in Southern Europe, North Africa and Asia provided
472 sufficient suitable habitats to maintain a substantial population (Cramp & Simmons 1983;
473 Harrison & Prentice 2003). Second, we did not detect any evidence for population
474 bottlenecks or expansions at mtDNA or microsatellite markers for the continental population.
475 Furthermore, the observed lack of an isolation-by-distance pattern supports the view that lack
476 of structure is caused by high contemporary gene flow.

477

478 Lack of genetic structure across large geographic distances is rare among terrestrial animals
479 and has only been described in a handful of insects and birds (Beveridge & Simmons 2006;
480 Estoup *et al.* 1996; Funk *et al.* 2007; Reudink *et al.* 2011; Verkuil *et al.* 2012). By contrast,
481 most other terrestrial species show at least modest genetic structure (Avice 2000). Based on
482 the breeding ecology we offer two explanations for the high gene flow. First, Kentish plovers
483 often breed in temporarily available habitats such as salt marshes, alkaline lakes and fish

484 ponds and the long breeding season (which lasts up to 5 months) provides opportunities for
485 several successful breeding attempts per year (Kosztolányi *et al.* 2009; Székely & Lessells
486 1993). Local breeding locations at temporal salt lakes are often unstable and only suitable for
487 a fraction of the available breeding time promoting mobility of the breeders. Unpredictable
488 and unstable habitats have been proposed to explain panmixia in Dawson's burrowing bees
489 *Amegilla dawsoni* (Beveridge & Simmons 2006). Second, resighting and genetic data suggest
490 high breeding dispersal particularly by females. During the reproductive season Kentish
491 plover females can breed at sites hundreds of kilometers apart which will prevent breeding
492 locations from differentiation (Székely & Lessells 1993).

493

494 The results of the sex-biased gene flow analyses are concordant with resighting data, and
495 suggest a prominent role for females to maintain high gene flow between breeding locations.
496 We found higher estimates for migration rates and lower genetic structure (i.e. lower number
497 of significant pairwise comparisons) for maternally inherited mtDNA than biparentally
498 inherited autosomal microsatellites. This is unexpected on purely population genetic grounds
499 because the N_e of mtDNA is smaller than the corresponding N_e of nuclear microsatellites, and
500 mtDNA genetic markers should therefore coalesce faster (Ballard & Whitlock 2004; Edwards
501 *et al.* 2005). The Bayesian phylogeny based on mtDNA was very shallow and branch support
502 was poor or not in agreement with geographic sample origin. Models that restricted the
503 mtDNA haplotypes to their geographic origin received less support than the unconstrained
504 model. Genetic differentiation of island populations followed a linear isolation-by-distance
505 pattern for autosomal markers, but not for the maternally inherited mtDNA marker.

506

507 In principle, the apparently higher Nm estimates for mtDNA may have been an artifact of
508 differences between nuclear and mtDNA. We think that this is unlikely to affect our
509 conclusion for three reasons. First, mutation rates of microsatellites are assumed to be higher
510 than for the mtDNA control region (Buehler & Baker 2005; Ellegren 2000) but immigration
511 rates (xN_m) in MIGRATE do not rely on mutation rates since they are calculated by
512 multiplying Θ (equivalent to N_e multiplied with mutation rate per site per generation) with the
513 mutation scaled immigration rate M (equivalent to the immigration rate divided by the
514 mutation rate per site per generation, Beerli 2010).

515 Second, selection regimes may differ between microsatellites and mtDNA. The
516 characteristics of the genetic markers that we used probably did not differ from those of
517 neutral markers. Microsatellites are generally assumed to be largely neutral markers and all
518 of the microsatellite markers we used were located in presumably non-coding regions
519 (Küpper *et al.* 2008). For the mtDNA Tajima's D values were nonsignificant suggesting that
520 selection is not operating on the D-loop in the Kentish plover.

521

522 Thirdly, differences in N_e between the maternally and biparentally inherited markers should
523 also not change our conclusion about female-biased gene flow. The assumption that N_e for
524 nuclear markers is about four times larger than for mtDNA holds only if the adult sex ratio is
525 1:1 (Wright *et al.* 2005). It is possible that this assumption of an equal adult sex ratio is
526 violated in polyandrous Kentish plovers. A recent study showed that in at least one
527 population sex-biased chick mortality leads to a strong adult male bias with more than six
528 males per female (Kosztolányi *et al.* 2011). No Kentish plover population with an adult
529 female bias is known and most bird populations appear to have a male skewed adult sex ratio
530 (Donald 2007). An adult male bias over the entire range of the species would further increase
531 our estimates for female-biased gene flow for mtDNA and autosomal marker comparisons
532 and therefore we regard our current estimates for the sex-bias as conservative.

533

534 The results of the comparison of genetic differentiation at the Z-chromosomal marker and the
535 autosomal markers provided further support for female-biased gene flow. Estimates for
536 genetic differentiation were higher for the Z-chromosomal marker than for the autosomal
537 markers. In an analogous investigation of sex-biased dispersal in humans Ségurel *et al.*
538 (2008) modeled the observed outcomes for genetic differentiation (measured as F_{ST}) for
539 comparisons between X-chromosomal and autosomal markers for differing population sex
540 ratios and sex-biased migration rates using Wright's infinite island model of population
541 structure. Using the observed sixfold excess of adult males in a Kentish plover breeding
542 population (Kosztolányi *et al.* 2011), and adjusting for the ZW system, the model suggests
543 female-biased gene flow as the most likely explanation for higher genetic differentiation of
544 Z-chromosomal markers than autosomal markers.

545

546 The previous results are based on comparisons involving estimates derived from a single
547 marker for Z chromosome and mtDNA. Such comparisons alone can be misleading because
548 single marker statistics will be strongly influenced by stochastic effects (Edwards *et al.*
549 2005). However, we also found support for female-biased gene flow from multilocus
550 analyses of sex-biased dispersal. Population differentiation and relatedness were marginally
551 higher for adult males than females of different geographically coherent clusters. Despite the
552 consistency of the sex-bias dispersal analysis across the three different marker comparisons
553 the bias appeared to be of only moderate magnitude. Moderate sex-biased dispersal can be
554 hard to detect particularly when sample sizes are small. Moreover, any bias will fade away in
555 subsequent generations when migrants have been integrated into the breeding population
556 (Goudet *et al.* 2002; Prugnolle & de Meeus 2002).

557

558 It is also possible that the sex-biased gene flow is reduced by male natal dispersal. Higher
559 natal dispersal by males has been reported in other polyandrous shorebirds (Clarke *et al.*
560 1997). We can only indirectly test natal dispersal using recruitment data since ringing
561 recoveries of Kentish plover juveniles are scarce. Recruitment in two Kentish plover
562 populations which were studied over a period of five or more years showed no sex bias: at
563 FDP a total of 16 males and 17 females that were ringed at hatching were recruited
564 subsequently (Amat *et al.* 2001), and similarly, at TUZ 32 male and 29 female recruits were
565 caught over five field seasons (T Székely, A Kosztolányi, C Küpper, unpublished results).
566 Based on the observed strong adult male bias in polyandrous populations the number of male
567 recruits is surprisingly low and concordant with male-biased natal dispersal.

568

569 Phylogeographic studies of *Charadrius* species seem to support a role of mating systems on
570 population genetic structure. The closely related snowy plover shares many breeding biology
571 characteristics with the Kentish plover such as multiple clutches, polygamy and nesting in
572 unstable habitats (e.g. Page *et al.* 1995; Warriner *et al.* 1986). A number of snowy plover
573 populations are well monitored and a wealth of resighting data has been accumulated over the
574 last decades. Both snowy plover males and females are highly site faithful and more than
575 95% of male and female chicks return to breed at their natal sites in subsequent years
576 (Stenzel *et al.* 2011). During the breeding season snowy plovers are mobile and may breed at

577 several locations up to 660 km (females) and 840 km (males) apart (Stenzel *et al.* 1994).
578 Consistently, snowy plovers do not exhibit genetic structure across their North American
579 continental range (Funk *et al.* 2007). Lack of genetic structure was also found in another
580 plover species with a multiple clutch system the mountain plover *C. montanus* (Oyler-
581 McCance *et al.* 2008). In contrast, moderate population structure has been observed on a
582 relatively small spatial scale in the monogamous piping plover *C. melodus* (Miller *et al.*
583 2010). Additional genetic studies of monogamous and low latitude breeders in this genus are
584 needed to examine the association between mating systems and population genetics.

585

586 The analysis and comparison of genetic diversity and gene flow provided further insights into
587 the phylogeography of Kentish plovers. Gene flow was asymmetric with higher rates from
588 the islands towards the mainland for the Macaronesian populations located in the Atlantic
589 Ocean. This pattern may be driven by size differences among different landmasses. The
590 Macaronesian island archipelagos are remote and relatively small. Therefore, the plovers
591 emigrating from the mainland westwards are unlikely to encounter them. By contrast, Eurasia
592 and Africa form a large continental land mass and therefore emigrating plovers from
593 Macaronesia that fly east will almost certainly reach the continent breeding sites if they are
594 able to cover the distance. The situation is different for the Asian islands where the bias in the
595 direction of gene flow is smaller. Farasan Islands are located close to the mainland (< 40 km)
596 whereas the East Asian islands of Japan, Taiwan and Okinawa are also relatively close to the
597 mainland coast and of larger size than the Macaronesian islands. Therefore more mainland
598 plovers are more likely to reach these East Asian islands than the Macaronesian islands.

599

600 Despite the overall female biased gene flow we observed an interesting switch of sex-biased
601 gene flow. Low mean values of mtDNA and higher mean values for microsatellites suggested
602 male biased gene flow from the mainland to the islands whereas strongly female biased gene
603 flow was observed from the islands to the mainland (Table 3). This apparent difference in
604 sex-biased gene flow could have two explanations. Firstly, mainland and island plovers may
605 differ in their dispersal behavior or capabilities with island females and mainland males
606 dispersing further and the opposite sex dispersing less. Sex differences in migratory behavior
607 are known from other shorebird species with males and females wintering at different

608 locations (Gill *et al.* 1995; Nebel *et al.* 2002) although it is not known whether these sex
609 differences are population specific. However, we think that in Kentish plovers this is unlikely
610 because mainland females but not males were observed at distant breeding sites (Székely &
611 Lessells 1993). Secondly, the apparent male bias could be an artifact since for the mainland
612 to island comparisons the confidence intervals for mtDNA were large and exceeded those of
613 the microsatellites. Further studies are needed to test the whether the apparent asymmetrical
614 sex-biased gene flow has a biological meaning.

615

616 Island populations exhibited lower genetic diversity than mainland sites. This pattern has
617 been found in many other taxa using different marker types (Frankham 1997). However, the
618 genetic differentiation of the island populations was surprising because plovers are excellent
619 dispersers, live in both marine and terrestrial habitats, and we observed no genetic structure
620 on the continent. Islands close to the mainland (< 100 km) were only poorly differentiated
621 whereas more remote islands were well differentiated. Therefore we conclude that large
622 ocean stretches provide effective physical barriers for gene flow in Kentish plovers. The
623 negligible genetic differentiation of island populations close to the continent may explain the
624 discrepancies of the results between the two Bayesian clustering approaches. However,
625 Bayesian clustering analyses clearly showed that the wintering population of Kentish plovers
626 sampled in Taiwan consisted of a mix of migrating plovers from the mainland and Taiwan
627 residents.

628

629 The old age of the island archipelagos - the youngest island group is more than 20 million
630 years old - prevented us from using geological data for calibration to time the island
631 colonization events by Kentish plovers. We found signs of recent population declines at the
632 Azores and Canary island populations (STM and FUV) and there was a similar although
633 nonsignificant trend for the Cape Verde population (CVM). Low sample sizes did not allow
634 us to test for population fluctuations at Porto Santo (PST), the fourth remote Macaronesian
635 location, where we only found a single breeding pair and two of the three Asian clusters (JPN
636 and OKN). As in other analyses, the nuclear markers were more informative in recovering
637 the demographic history than the mtDNA marker.

638

639 In conclusion, we found no genetic structure in Kentish plovers on a continental scale. By
640 contrast, island populations were moderately differentiated from the mainland population and
641 genetic differentiation increased with ocean distance that separated breeding locations. A
642 comparison of differentiation and migration rates between mtDNA, a Z-linked microsatellite
643 marker and 21 microsatellite markers suggests that high gene flow is mediated through
644 dispersal of breeding females. Future work should focus on the effects of the mating system
645 and reproductive biology on genetic differentiation in this taxonomic group.

646

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- 864
- 865

865 Table 1. Details of geographic locations and sample sizes for mitochondrial and microsatellite markers of 21 Kentish plover sites sampled.

Site	Country	Abbreviation	Latitude	Longitude	Category	Status	Year	N_{mito}	N_{micro}
Santa Maria	Azores/Portugal	STM	36° 58'N	25° 06'W	I	B	2009	16	25
Boa Vista / Sal	Cape Verde	CVB	15°56'– 16°48'N	22°59'– 22°40'W	I	B	2007	3	11
Maio	Cape Verde	CVM	15°09'N	23°13'W	I	B	2007-08	12	25
Fuerteventura	Canary Islands/Spain	FUV	28°26'N	14°00'W	I	B	2009	17	25
Porto Santo	Madeira Islands/Portugal	PST	33°04'N	16° 21'W	I	B	2009	2	2
Samouco	Portugal	SAM	38°43'N	09°00'W	M	B	2009	17	25
Gharifa	Morocco	GHR	35°09'– 35°34'N	05°59'– 06°07'W	M	B	2009	12	11
Doñana	Spain	DON	36°56'N	06°21'W	M	B	2004	17	25
Fuente de Piedra	Spain	FDP	37°06'N	04°45'W	M	B	2006	17	25
Beltringharder Koog	Germany	BLK	54°32'N	08°54'E	M	B	2009	10	13
Kujalnik	Ukraine	KUJ	46°45'N	30°36'E	M	B	2006	17	15
Tuzla	Turkey	TUZ	36°42'N	35°03'E	M	B	2004	16	25
Farasan Islands	Saudi Arabia	FAR	16°48'N	41°53'E	I	B	2007-08	16	25
Lake Elton	Russia	ELT	49°12'N	46°39'E	M	B	2006-07	16	14
Al Wathba	United Arab Emirates	ALW	24°16'N	54°36'E	M	B	2005-06	16	25
Xinjiang	China	XIN	44°50'– 47°39'N	83°02'– 87°31'E	M	B	2008	9	7
Bohai	China	BOH	39°06'N	118°11'E	M	B	2009	5	5
Taiwan	Taiwan	TWB	24°30'N	120°40'E	I	B	2005-06	10	25
Taiwan	Taiwan	TWW	24°30'N	120°40'E	I	W	2004-07	8	22
Okinawa	Japan	OKN	26°11'N	127°43'E	I	B	2006-07	3	3
Japan	Japan	JAP	35°52'N	140°45'E	I	B	2004-09	6	7

866 N_{mito} , number of individuals for which a part of the control region was sequenced; N_{micro} , number of individuals genotyped at microsatellite loci;867 I , island; M , mainland; B , breeding population; W , wintering population

868

868 Table 2. Genetic diversity of 20 breeding locations for Kentish plover measured by 427 bp fragment of the mitochondrial control region and 21
 869 autosomal microsatellite markers

Population	mtDNA			Microsatellites					
	n_{HT}	h	π	D_T	A	A_{rich}	H_o	H_e	P_{TPM}
<i>Island</i>									
STM	2	0.13	0.0002	-1.16	4.9	2.53	0.66	0.66	0.002
CVB	2	0.67	0.0016	na	5	2.53	0.63	0.69	0.065
CVM	3	0.62	0.0054	1.44	6	2.53	0.63	0.68	0.20
FUV	4	0.71	0.0033	0.59	8.2	2.85	0.76	0.77	0.007
PST	1	0	0	na	1.8	2	0.57	0.50	na
FAR	5	0.71	0.0039	0.42	8	2.77	0.71	0.76	0.39
TWB	7	0.95	0.0078	-0.80	8.3	2.8	0.72	0.76	0.34
OKN	2	0.67	0.0031	na	3.2	2.66	0.80	0.70	na
JAP	3	0.60	0.0045	na	5.3	2.81	0.76	0.77	na
<i>Mainland</i>									
SAM	8	0.77	0.0039	-0.7	9.6	2.94	0.76	0.78	0.45
GHR	5	0.67	0.0039	-0.59	6.4	2.82	0.74	0.74	0.52
DON	8	0.85	0.004	-0.58	9.7	3.03	0.76	0.79	0.29
FDP	5	0.81	0.0042	0.68	9.4	2.95	0.78	0.79	0.73
BLK	5	0.76	0.004	-0.18	7.2	2.83	0.74	0.77	1
KUJ	11	0.91	0.0059	-0.85	8.0	2.95	0.75	0.80	0.07
TUZ	8	0.83	0.0042	-0.51	10.3	3.11	0.79	0.81	0.87
ELT	11	0.95	0.0071	-0.34	7.9	2.99	0.81	0.80	0.22
ALW	8	0.86	0.0049	-0.46	9.8	2.95	0.81	0.79	0.68
XIN	6	0.83	0.0059	-0.68	5.6	2.8	0.69	0.79	na
BOH	2	0.60	0.0014	na	5.2	3	0.73	0.80	na
All Mainland	41	0.83	0.0047	-1.66	15.6	2.89	0.77	0.80	0.36

870 n_{HT} , number of haplotypes; h , haplotype diversity; π , nucleotide diversity; D_T , Tajima's D; A , number of alleles; A_{rich} , allelic richness; H_o ,
 871 observed heterozygosity; H_e , expected heterozygosity; P_{TPM} , p-value for tests of heterozygosity deficiency and excess using the two-phased
 872 mutation model in BOTTLENECK

873 Table 3. Estimates of Θ and Nm for genetic clusters of Kentish plovers estimated from 21 autosomal microsatellite markers and a 427 bp
874 fragment of the mitochondrial D-loop using MIGRATE. Due to differences in the mode of inheritance, Θ and Nm values estimated from
875 microsatellites were divided by four to make them comparable with corresponding values from mitochondrial DNA under the assumption of
876 equal sex ratios. Migration rates were averaged over two converged independent runs with five replicates. Modal values with 95% confidence
877 limits in parentheses are given.

Population	Θ	Cape Verde →	Santa Maria →	Fuerteventura →	Mainland →	Farasan Islands→	East Asian Islands →
<i>MtDNA</i>							
Cape Verde	0.003 (0–0.1)		0.3 (0–14)	0.3 (0–14)	0.3 (0–14)	0.3 (0–14)	0.3 (0–14)
Santa Maria	0.003 (0–0.1)	0.3 (0–14.3)		0.3 (0–14)	0.3 (0–14.3)	0.3 (0–14.3)	0.3 (0–14.3)
Fuerteventura	0.003 (0–0.1)	0.3 (0–14.7)	0.3 (0–14.7)		0.3 (0–14.7)	0.3 (0–14.7)	0.3 (0–14.7)
Mainland	0.1 (0–0.4)	37.7 (0–183)	21.3 (0–146.7)	22.7 (0–166)		13.3 (0–129.3)	23.7 (0–138.7)
Farasan Islands	0.003 (0–0.1)	0.3 (0–16.3)	0.3 (0–17.3)	0.3 (0–16.7)	0.3 (0–16.7)		0.3 (0–16.3)
East Asian Islands	0.003 (0–0.1)	0.3 (0–18)	0.3 (0–19)	0.3 (0–18.7)	0.3 (0–20.3)	1 (0–19)	
<i>Microsatellites</i>							
Cape Verde	0.008 (0–0.5)		0.1 (0–3.8)	0.1 (0–3.8)	4.1 (0–8)	0.1 (0–3.8)	0.1 (0–3.9)
Santa Maria	0.008 (0–0.4)	0.1 (0–3.7)		0.1 (0–3.7)	10 (0–6.5)	0.1 (0–3.7)	0.1 (0–3.7)
Fuerteventura	0.3 (0–0.7)	0.1 (0–4.8)	0.1 (0–4.5)		5.1 (0.6–9.3)	0.1 (0–4.25)	0.1 (0–4.8)
Mainland	2.3 (1.7–3)	10 (5.3–14.5)	6.8 (2.3–11.3)	6.8 (2.2–11.2)		6.8 (2.1–11.2)	8.9 (4.3–13.4)
Farasan Islands	0.3 (0–0.7)	0.4 (0–5.4)	0.1 (0–5)	0.1 (0–4.3)	5.9 (1.3–10.3)		0.1 (0–7.1)
East Asian Islands	0.3 (0–0.7)	2.3 (0–6.5)	0.1 (0–5.2)	0.1 (0–5.3)	6.4 (3.2–12.6)	0.1 (0–4.9)	

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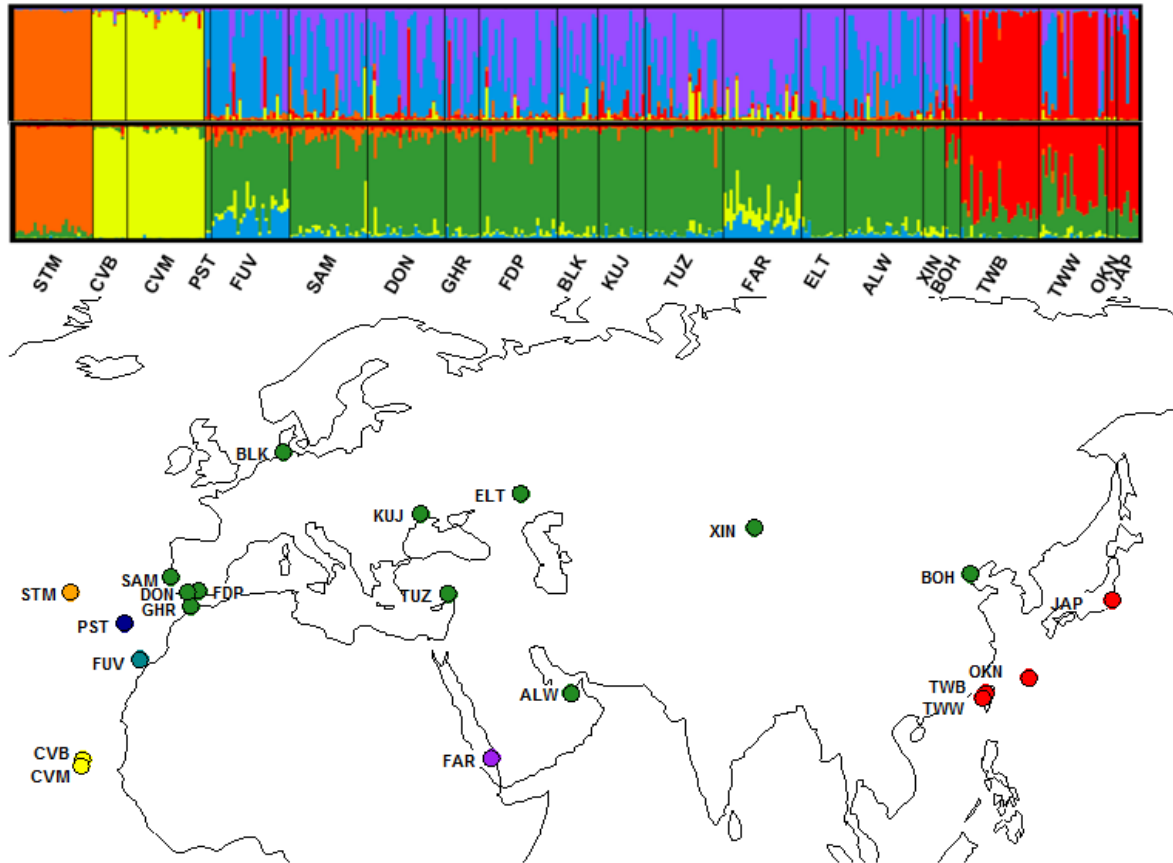
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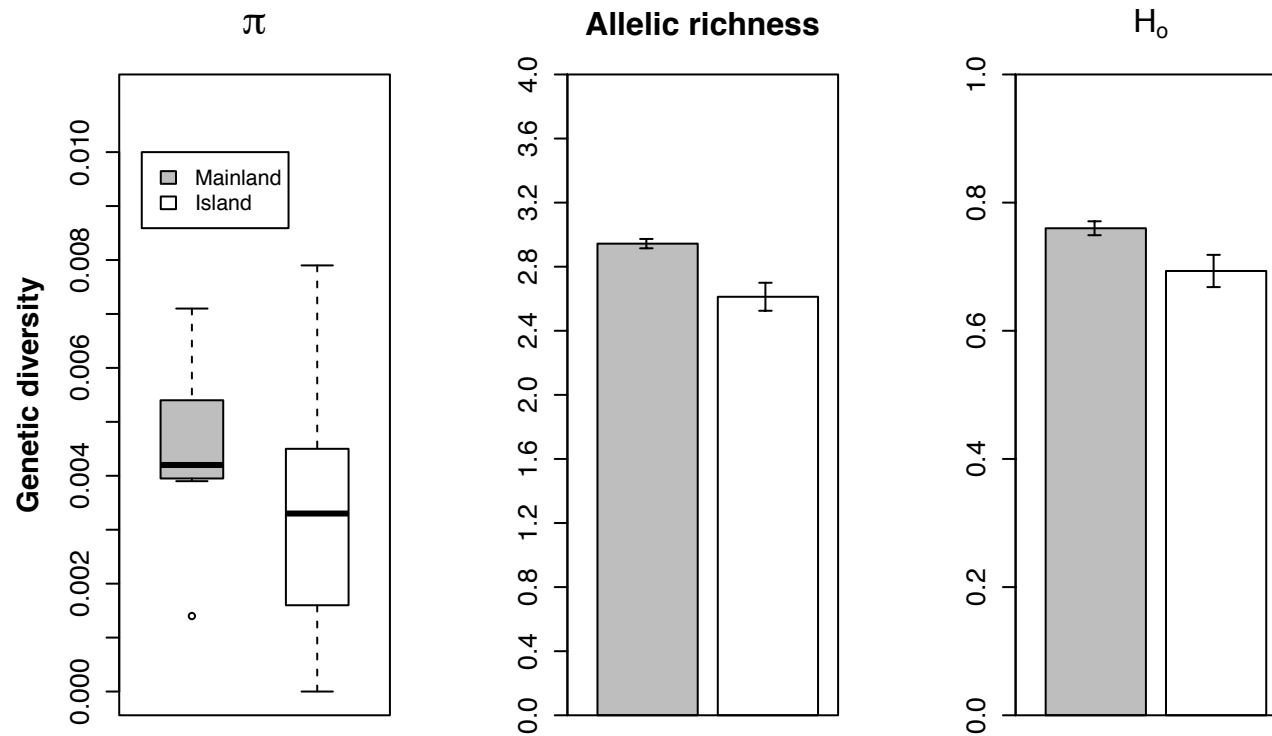


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886 Figure 1. Map of sampling locations of 21 Kentish plover populations, and assignment into population clusters using STRUCTURE (top diagram) and TESS
887 (lower diagram). Both programs suggested five clusters as the most likely value for K. There was disagreement of assignment of Kentish plovers of three
888 island populations PST, FUV and FAR (blue, turquoise and purple circles on map). According to STRUCTURE, plovers from mainland sites had genotypes
889 intermediate between island clusters FUV (blue) and FAR (purple) whereas plovers from PST showed genotypes intermediate between FUV and the East

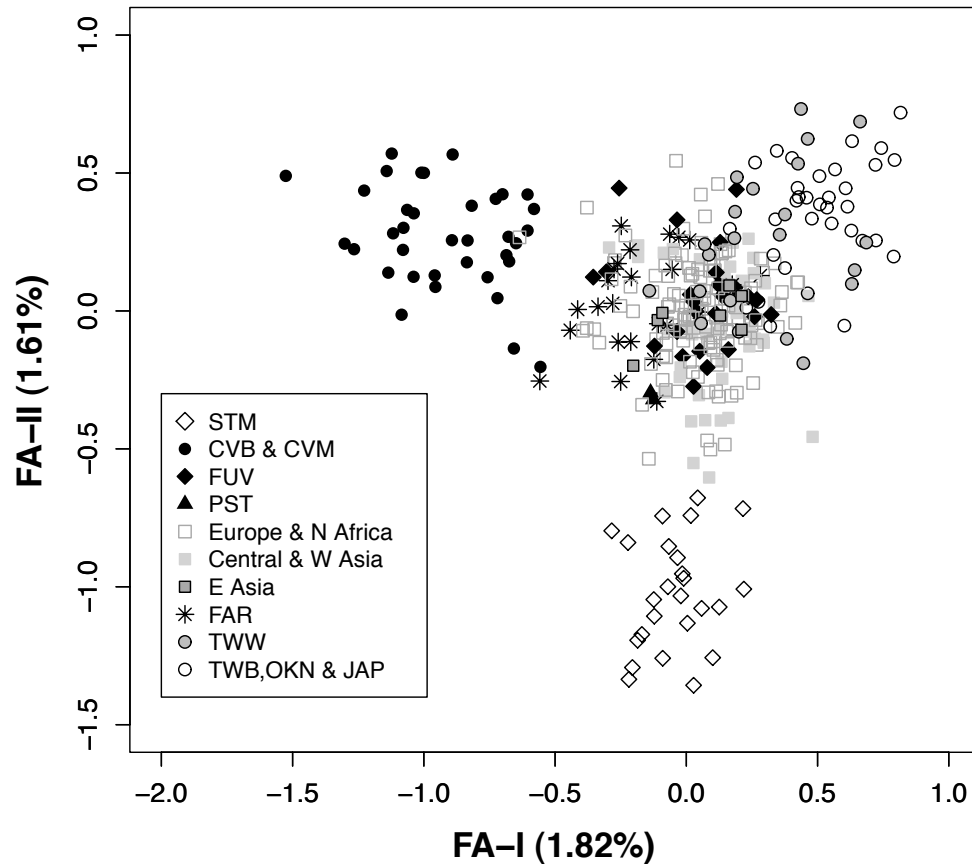
890 Asian Islands locations. According to TESS, plovers from PST were not different from mainland breeders, and plovers from FUV and FAR largely resembled
891 mainland plovers but showed signs of incipient genetic differentiation.

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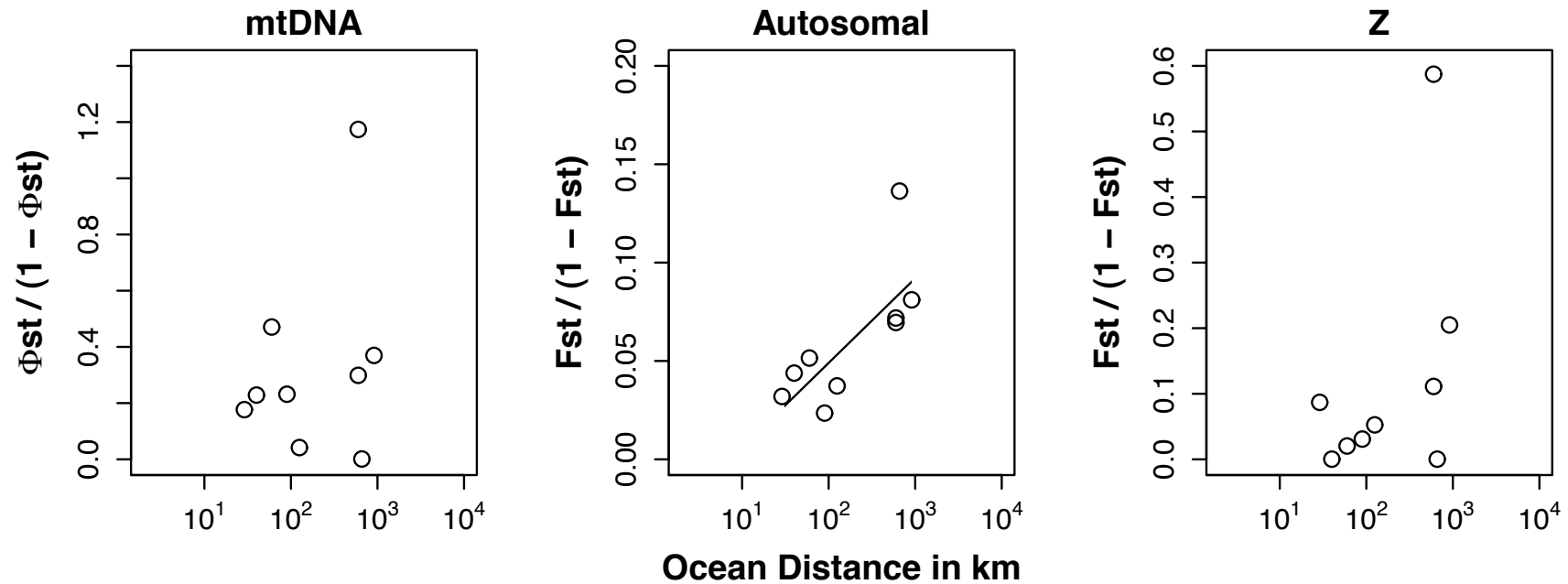
894 Figure 2. Genetic diversity of nine island and eleven mainland breeding locations of Kentish plovers. There was no significant difference in mitochondrial
895 sequence diversity π , but mainland breeding locations harbored higher nuclear genetic diversity (allelic richness and observed heterozygosity H_0) than island
896 breeding locations based on 21 autosomal microsatellite markers. Median given for π , mean \pm standard error given for allelic richness and H_0 .



899 Figure 3. Genetic differentiation of Kentish plover populations visualized with a Factorial Correspondence Analysis. Island populations are presented with
 900 open or filled black symbols. Gray squares refer to plovers sampled during the breeding season at eleven mainland sites. Europe and North Africa includes

901 samples from SAM, DON, FDP, GHR, BLK and KUJ, Central and W Asia includes samples from TUZ, ALW and ELT, and Eastern Asia includes samples
902 from XIN and BOH.

903



904

905 Figure 4. Relationship between genetic differentiation and distance over open ocean of nine island locations vs the mainland for mitochondrial DNA,
906 autosomal microsatellites and a Z chromosomal microsatellite marker. Only autosomal microsatellites showed a significant linear relationship with distance.

1

2 Supporting Information

3 Table S1. Pairwise F_{ST} (above diagonal) and R_{ST} values (below diagonal) for 20 breeding locations and one wintering location of Kentish plover
 4 based on 21 autosomal microsatellites. Island breeding populations are marked by an asterisk. Negative values represent computation
 5 idiosyncrasies and are effectively zero. We tested for significance using 1000 random permutations. Significant values at $P < 0.01$ and $q < 0.01$
 6 are presented in bold; remaining values are all nonsignificant.

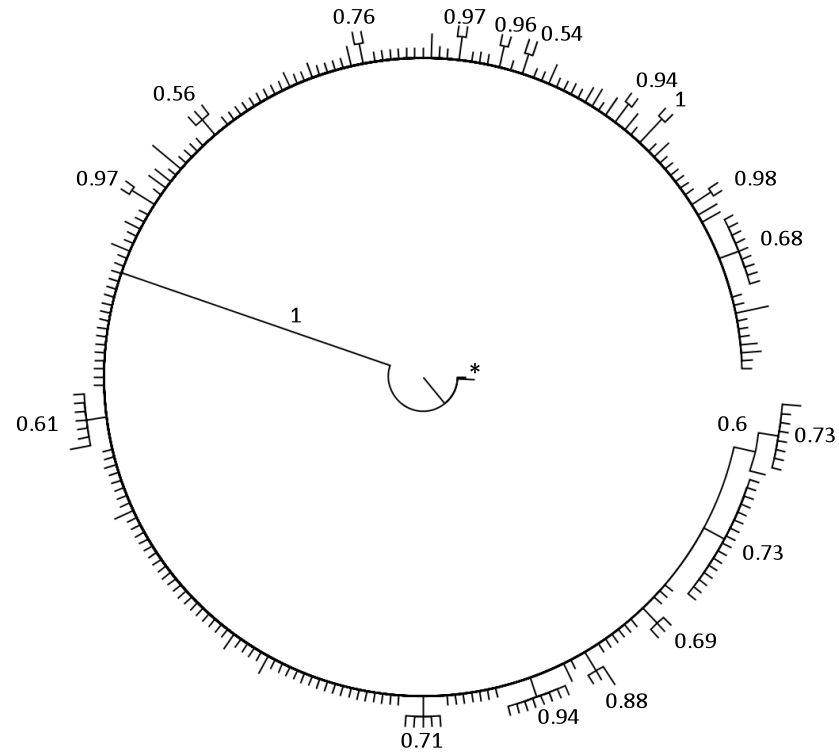
Site	STM*	CVB*	CVM*	FUV*	PST*	SAM	GHR	DON	FDP	BLK	KUJ	TUZ	FAR*	ELT	ALW	XIN	BOH	TWB*	TWW	OKN*	JAP*
STM*	-	0.16	0.17	0.10	0.18	0.08	0.11	0.08	0.09	0.11	0.09	0.08	0.13	0.09	0.08	0.07	0.11	0.13	0.11	0.17	0.15
CVB*	0.26	-	0.01	0.08	0.23	0.07	0.10	0.07	0.07	0.07	0.07	0.06	0.08	0.08	0.07	0.07	0.09	0.12	0.08	0.13	0.12
CVM*	0.22	0.02	-	0.08	0.24	0.08	0.11	0.07	0.07	0.06	0.08	0.07	0.07	0.10	0.07	0.09	0.08	0.11	0.08	0.14	0.13
FUV*	0.11	0.12	0.11	-	0.14	0.02	0.05	0.03	0.03	0.03	0.02	0.03	0.06	0.02	0.03	0.01	0.04	0.05	0.03	0.09	0.07
PST*	0.34	0.41	0.47	0.31	-	0.11	0.17	0.13	0.14	0.15	0.10	0.11	0.13	0.11	0.12	0.12	0.16	0.18	0.16	0.26	0.21
SAM	0.04	0.09	0.13	0.03	0.20	-	0.02	0	0.01	0.02	0	0.01	0.04	0	0.01	-0.01	0.02	0.04	0.02	0.07	0.07
GHR	0.09	0.16	0.22	0.10	0.18	0.03	-	0.02	0.03	0.02	0.02	0.02	0.06	0.03	0.02	0.03	0.02	0.06	0.04	0.07	0.06
DON	0.07	0.11	0.16	0.04	0.18	0.01	-0.01		0.01	0	0	0	0.04	0	0	-0.01	0.01	0.04	0.02	0.06	0.04
FDP	0.10	0.05	0.11	0.04	0.17	0.01	0.03	0.01	-	0.01	0.01	0.01	0.03	-0.01	0	-0.01	0.02	0.05	0.02	0.05	0.05
BLK	0.15	0.13	0.16	0.04	0.20	0.04	0.02	0.01	0.03	-	0.01	0.01	0.03	0	0.01	0.01	0.01	0.04	0.02	0.05	0.05
KUJ	0.11	0.09	0.17	0.08	0.12	0.02	-0.01	0.02	0	0.02	-	0	0.03	0	0	-0.01	0	0.03	0.02	0.05	0.05
TUZ	0.08	0.07	0.13	0.06	0.17	0.01	0	0	0	0.03	0	-	0.04	0	0.01	-0.01	0.01	0.03	0.02	0.04	0.04
FAR*	0.13	0.03	0.06	0.03	0.20	0.02	0.04	0.03	0.01	0.02	0.02	0.02	-	0.02	0.03	0.03	0.05	0.07	0.04	0.06	0.08
ELT	0.12	0.07	0.14	0.06	0.15	0.02	-0.01	0	-0.01	0.01	-0.02	0	0	-	0	-0.01	0.01	0.03	0.02	0.05	0.05
ALW	0.09	0.06	0.10	0.03	0.22	0.01	0.02	-0.01	0	0.01	0.01	0	0.01	0	-	-0.01	0.03	0.04	0.02	0.05	0.05
XIN	0.06	0.14	0.20	0.06	0.21	-0.01	-0.05	-0.03	0	0.04	-0.01	-0.01	0.03	-0.01	0	-	0	0.03	0.01	0.08	0.03
BOH	0.09	0.06	0.13	0.03	0.20	0	-0.05	-0.03	-0.03	-0.01	-0.03	-0.04	0	-0.02	-0.04	-0.03	-	0.03	0.02	0.06	0.03
TWB*	0.13	0.12	0.14	0.05	0.29	0.05	0.04	0.03	0.04	0.07	0.06	0.05	0.05	0.05	0.02	0.02	0.01	-	0.01	0.06	0.05
TWW	0.15	0.08	0.10	0.04	0.31	0.04	0.05	0.03	0.02	0.03	0.05	0.04	0.02	0.03	0	0.02	-0.01	0	-	0.03	0.02
OKN*	0.15	0.22	0.25	0.07	0.25	0.07	0.06	0.07	0.07	0.08	0.04	0.09	0.06	0.05	0.06	0.06	0.04	0.04	0.06	-	0.03
JAP*	0.10	0.20	0.25	0.10	0.21	0.08	0	0.02	0.06	0.05	0.05	0.04	0.10	0.01	0.04	0.01	-0.01	0.05	0.07	0.02	-

7

8

8 Table S2. Pairwise Φ_{ST} values based on a mitochondrial marker (above diagonal) and pairwise F_{ST} values based on a Z-linked microsatellite
9 marker (below diagonal, calculated with males only) for 20 breeding locations and one wintering location of Kentish plover. Island breeding
10 populations are marked by an asterisk. Negative values represent computation idiosyncrasies and are effectively zero. We tested for significance
11 using 1000 random permutations. Significant values at $P < 0.01$ and $q < 0.01$ are presented in bold; remaining values are all nonsignificant.

Site	STM*	CVB*	CVM*	FUV*	PST*	SAM	GHR	DON	FDP	BLK	KUJ	TUZ	FAR*	ELT	ALW	XIN	BOH	TWB*	TWW	OKN*	JAP*
STM*	-	0.92	0.45	0.27	0.94	0.37	0.49	0.41	0.37	0.58	0.29	0.33	0.30	0.42	0.54	0.49	0.86	0.30	0.56	0.65	0.52
CVB*	0.47	-	0.32	0.62	0.90	0.60	0.62	0.61	0.59	0.65	0.45	0.58	0.53	0.44	0.62	0.53	0.84	0.41	0.60	0.70	0.61
CVM*	0.29	0.16	-	0.32	0.32	0.23	0.25	0.25	0.27	0.30	0.17	0.23	0.22	0.13	0.22	0.23	0.39	0.18	0.29	0.33	0.30
FUV*	0.13	0.51	0.24	-	0.44	0.17	0.23	0.20	0.19	0.28	0.16	0.16	0.21	0.26	0.34	0.24	0.38	0.15	0.27	0.30	0.26
PST*	-0.09	0.57	0.06	-0.36	-	0.02	-0.10	-0.06	0.02	-0.17	-0.06	0.03	0.32	-0.13	-0.09	-0.18	-0.02	-0.08	-0.17	0.71	0.34
SAM	0.20	0.46	0.10	0.03	-0.27	-	-0.03	-0.05	-0.02	0.00	-0.02	-0.04	0.13	0.05	0.10	-0.02	0.04	-0.01	0.01	0.34	0.18
GHR	0.22	0.30	-0.02	0.14	-0.13	0.01	-	-0.04	-0.01	-0.03	-0.02	-0.02	0.14	0.04	0.07	-0.06	0.01	-0.01	-0.01	0.37	0.20
DON	0.06	0.39	0.15	-0.01	-0.32	0.03	0.07	-	-0.03	-0.04	-0.01	-0.02	0.14	0.05	0.09	-0.02	0.00	0.01	0.00	0.36	0.20
FDP	0.19	0.44	0.11	0.06	-0.21	-0.03	0.01	0.06	-	-0.02	0.01	-0.01	0.15	0.07	0.14	0.00	0.06	0.00	0.03	0.33	0.18
BLK	0.15	0.30	0.06	0.10	-0.18	0.05	-0.01	0.03	0.02	-	0.01	0.02	0.22	0.03	0.08	-0.02	-0.03	0.03	-0.02	0.43	0.24
KUJ	0.16	0.48	0.12	-0.02	-0.35	-0.07	0.01	-0.01	-0.04	0.03	-	-0.01	0.09	0.04	0.09	-0.01	0.04	0.00	0.01	0.24	0.12
TUZ	0.16	0.44	0.14	0	-0.31	-0.03	0.04	0.01	-0.01	0.03	-0.06	-	0.12	0.06	0.11	0.01	0.05	0.01	0.02	0.30	0.16
FAR*	0.23	0.20	0.01	0.20	-0.03	0.10	-0.01	0.10	0.07	-0.01	0.10	0.11	-	0.21	0.28	0.18	0.30	0.11	0.20	0.29	0.23
ELT	0.24	0.63	0.18	0.05	-0.23	-0.06	0.04	0.08	-0.05	0.09	-0.07	-0.03	0.14	-	0.02	0.02	0.04	0.06	0.05	0.30	0.20
ALW	0.12	0.33	0.08	0.02	-0.27	0	0.01	-0.01	0	-0.04	-0.02	-0.02	0.04	0.04	-	0.05	0.11	0.12	0.09	0.42	0.29
XIN	0.35	0.68	0.18	0.23	0.09	0.05	0.02	0.22	0.01	0.13	0.07	0.10	0.14	-0.02	0.14	-	-0.04	-0.03	-0.02	0.29	0.18
BOH	0.15	0.47	0.09	0.04	-0.30	-0.04	-0.06	0	-0.06	-0.03	-0.05	-0.03	-0.01	-0.04	-0.02	-0.02	-	0.02	-0.06	0.66	0.36
TWB*	0.11	0.49	0.24	-0.03	-0.34	0.05	0.14	-0.02	0.08	0.10	0	0.02	0.18	0.08	0.03	0.24	0.03	-	-0.02	0.11	0.06
TWW	0.14	0.43	0.15	0.02	-0.28	0	0.04	0	0	0.04	-0.02	0	0.07	0	0.01	0.10	-0.09	0.01	-	0.30	0.07
OKN*	0.02	0.52	0.21	-0.05	-0.43	0.05	0.09	-0.12	0.06	0	0	0	0.06	0.13	-0.04	0.31	-0.09	-0.10	-0.08	-	-0.05
JAP*	0.08	0.45	0.13	0.04	-0.33	0.06	-0.02	-0.03	0.01	-0.15	0.02	0	-0.01	0.11	-0.08	0.16	-0.13	0.03	-0.02	-0.13	-



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14 Figure S1. Bayesian phylogeny based on a 427 bp mitochondrial DNA control region fragment of 245 Kentish plovers with three snowy plovers
 15 as outgroup (indicated by asterisk). Only five nodes are well supported (>0.95) and the topology is poorly associated with geographic
 16 distribution of the haplotypes. Two alternative topologies (not shown) constrained by geographic origin of the samples received little support.

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