High gene flow on a continental scale in the polyandrous Kentish plover *Charadrius alexandrinus*

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Abstract

Gene flow promotes genetic homogeneity of species in time and space. Gene flow can be modulated by sex-biased dispersal that links population genetics to mating systems. We investigated the phylogeography of the widely distributed Kentish plover *Charadrius alexandrinus*. This small shorebird has a large breeding range spanning from Western Europe to Japan and exhibits an unusually flexible mating system with high female breeding dispersal. We analysed genetic structure and gene flow using a 427-bp fragment of the mitochondrial (mtDNA) control region, 21 autosomal microsatellite markers and a Z microsatellite marker in 397 unrelated individuals from 21 locations. We found no structure or isolation-by-distance over the continental range. However, island populations had low genetic diversity and were moderately differentiated from mainland locations. Genetic differentiation based on autosomal markers was positively correlated with distance between mainland and each island. Comparisons of uniparentally and biparentally inherited markers were consistent with female-biased gene flow. Maternally inherited mtDNA was less structured, whereas the Z-chromosomal marker was more structured than autosomal microsatellites. Adult males were more related than females within genetic clusters. Taken together, our results suggest a prominent role for polyandrous females in maintaining genetic homogeneity across large geographic distances.

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

Introduction

Investigating the link between ecology and evolution is a central challenge of population biology. Dispersal has a strong influence on gene flow, genetic diversity and population structure which may in turn affect the efficiency of selection and local adaptation (Bohonak 1999; Clobert *et al.* 2004). However, dispersal is a complex process that is often difficult to assess (Edwards 1993; Okamura & Freeland 2002). For each individual, the motivation to disperse often depends on age (i.e. natal or breeding dispersal) and may differ between sexes. Sex-biased dispersal has been related to mating

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systems, resource competition and inbreeding avoidance (Greenwood 1980; Lawson Handley & Perrin 2007). In socially monogamous species such as many birds, local resource competition among related females is predicted to lead to female-biased dispersal, whereas in polygynous species such as many mammals, local mate competition among related males should lead to male dispersal (Greenwood 1980; Clarke *et al.* 1997; Lawson Handley & Perrin 2007). A review of mark–recapture studies in birds suggested that dispersal is predominantly female biased, although many species showed no sex bias and only few studies showed male-biased dispersal (Clarke *et al.* 1997). However, sex-biased dispersal does not necessarily lead to sex-biased gene flow because it is often not clear whether dispersers are able to successfully breed and contribute to the gene pool at their new location (Prugnolle & de Meeus 2002).

The results of studies of sex-biased gene flow have challenged simplistic views on associations of sex-biased dispersal with mating systems. In mammals, contrary to the predictions from mating system theory, female dispersal is found in many polygynous species, particularly in primates, whereas male dispersal also occurs in a number of monogamous mammals (Lawson Handley & Perrin 2007). In birds, few genetic studies have demonstrated female-biased gene flow (Piertney *et al.* 2000; Johnson *et al.* 2003; Bouzat & Johnson 2004; Wright *et al.* 2005; Rönkä *et al.* 2008, 2012) and male-biased gene flow is reported from a similarly small number of bird species (e.g. Edwards 1993; Gibbs *et al.* 2000; Scribner *et al.* 2001; Mäki-Petäys *et al.* 2007; Capparoz *et al.* 2009; Hefti-Gautschi *et al.* 2009; Liu *et al.* 2012). Importantly, in some birds, male-biased gene flow was even found when recapture data suggested otherwise (Li & Merilä 2010).

Several approaches have been developed to examine sex-biased dispersal using molecular markers. Studies have compared estimates for population differentiation and migration rates between autosomal microsatellites and sex-specific markers (e.g. markers from nonrecombining chromosomal segments of the Y chromosome or mitochondrial (mt) DNA, e.g. Seielstad *et al.* 1998; Wright *et al.* 2005; Douadi *et al.* 2007; Lawson Handley & Perrin 2007). The rationale for the latter approach is that the uniparentally inherited marker is shaped only by the demographic history of the sex carrying the marker. Differences in estimates of population structure or gene flow between uniparentally and biparentally inherited markers may therefore reveal different genetic contributions by the sexes. For species in which females are dispersing and males are philopatric, genetic differentiation is expected to be highest at Y-chromosomal markers, followed by autosomal markers and mtDNA. However, an examination of sex-biased gene flow based only on differences between biparentally and uniparentally inherited markers makes it difficult to disentangle sex-biased dispersal from differences in marker characteristics such as effective population sizes (which for uniparentally inherited markers is ¼ that of autosomal markers in diploid monogamous systems), mutation rates or selection operating on these markers. Additionally, mtDNA is often subject to bouts of natural selection, making inferences of effective population size from standing levels of genetic diversity within populations challenging (reviewed by Ballard & Whitlock 2004; Dowling *et al.* 2008; Edwards & Bensch 2009).

To overcome these problems, two alternatives have been proposed. First, sex-biased dispersal may be inferred from comparisons of summary statistics between biparentally inherited autosomal markers and biparentally inherited markers such as X- or Z-chromosomal markers that spend more time in one sex than the other one (Ségurel *et al.* 2008; Carling *et al.* 2010; Li & Merilä 2010). X (Z)-chromosomal markers undergo recombination as do autosomal markers, but females (males) carry two-thirds of the X (Z)-specific variation. Comparisons between X (Z) markers and autosomal markers to examine sex-specific gene flow provide an improvement over comparisons involving mtDNA because the differences in effective population sizes are less pronounced (the effective population size of X (Z)-chromosomal markers is ¾ that of autosomal markers). Second, sex-biased dispersal can be inferred by comparing sex-specific summary statistics such as F_{ST}/F_{IS} values and relatedness estimates calculated for each sex separately when individuals are sampled after the dispersal event (Goudet *et al.* 2002; Prugnolle & de Meeus 2002). This approach largely overcomes the problems caused by different effective population sizes, mutation rates and selection pressures. However, this approach may only detect strong and instantaneous biases because the signal is lost immediately when gene flow is followed by successful reproduction. This is because the offspring will inherit randomly chosen maternal and paternal alleles, thereby destroying any sex-specific pattern of differentiation built-up in the previous generation (Prugnolle & de Meeus 2002).

Here, we investigate the patterns of genetic diversity, population differentiation and sex-biased gene flow in a small shorebird, the Kentish plover *Charadrius alexandrinus*. This species has an unusually large geographic range including Northern Africa, Europe and Asia (Cramp & Simmons 1983). Some populations breed on isolated ocean archipelagos such as Macaronesia (Azores, Canary Islands, Cape Verde Islands, Madeira), and their geographic isolation may reduce exchange of migrants (del Hoyo *et al.* 1996). Many Kentish plovers

are polygamous and have multiple clutches with one parent—usually (but not always) the female—abandoning the brood to re-mate, while the remaining parent provides care for the chicks until the chicks are independent (Lessells 1984; Székely & Lessells 1993; Amat *et al.* 1999; Székely *et al.* 1999, 2006; Kosztolányi *et al.* 2009). The deserting female may then move large distances between different breeding attempts (Székely & Lessells 1993). This female-biased breeding dispersal may create high sex-biased gene flow between breeding locations.

We sampled several thousand kilometres across the breeding range of the Kentish plover, including eleven mainland and ten island populations. We had three objectives. First, we compared patterns of genetic diversity between mainland and island populations and looked for signals of recent population size changes. Second, we investigated the extent of genetic differentiation by including samples from breeding sites across most of its breeding range. Third, we examined whether gene flow is principally driven by dispersing polyandrous females during the breeding season. Because of the problems associated with the various approaches to estimate sex-biased dispersal (Prugnolle & de Meeus 2002), we tested the hypothesis of female-mediated gene flow using three different approaches to compare genetic differentiation and migration rates between mitochondrial DNA, 21 autosomal and a Z-chromosomal microsatellite marker. We predicted (i) lower genetic differentiation and higher migration rates for mtDNA than autosomal markers, (ii) stronger genetic differentiation for the Z-chromosomal marker than for autosomal markers, (iii) lower genetic differentiation and relatedness among adult females than males.

Material and methods

Sampling and molecular analyses

We obtained DNA samples from 397 presumably unrelated adults or chicks of 21 Kentish plover populations (20 breeding and one wintering population) in Africa and Eurasia (Table 1, Fig. 1). Three samples of the closely related snowy plover *Charadrius nivosus* sampled at Bahía de Ceuta, Mexico (23°54′N, 106°57′ W), were included as an out-group for phylogenetic analyses.

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	2007–2008	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	2007–2008	16	25
Lake Elton	Russia	ELT	49°12′N	46°39′E	M	B	2006–2007	16	14
Al Wathba	United Arab Emirates	ALW	24°16′N	54°36′E	M	B	2005–2006	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	2005–2006	10	25
Taiwan	Taiwan	TWW	24°30′N	120°40′E	I	W	2004–2007	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	2004–2009	6	7

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

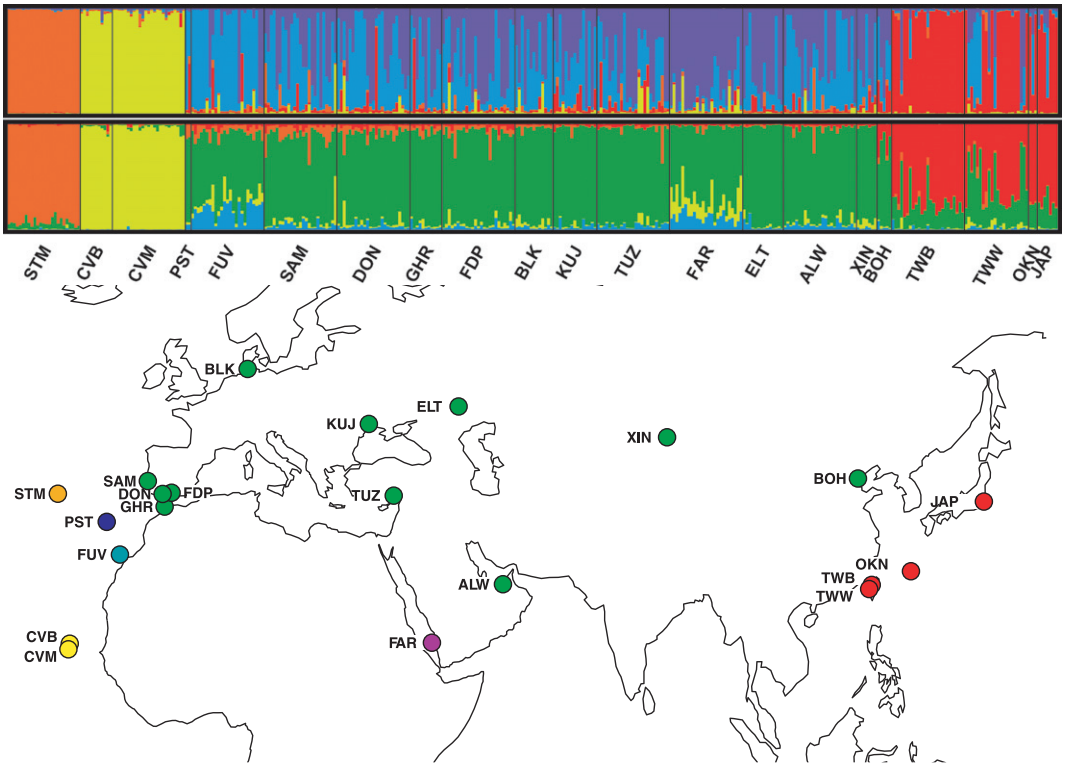

Fig. 1 Map of sampling locations of 20 Kentish plover breeding populations and one wintering locations (TWW) with their assignment into population clusters using STRUCTURE (top diagram) and TESS (lower diagram). Both programs suggested five clusters as the most likely value for *K*. There was disagreement of assignment of Kentish plovers from three island populations PST, FUV and FAR (blue, turquoise and purple circles on map, respectively). According to STRUCTURE, plovers from mainland sites had genotypes intermediate between island clusters FUV (blue) and FAR (purple), whereas plovers from PST showed genotypes intermediate between FUV and the East Asian Islands locations. According to TESS, plovers from PST were not different from mainland breeders, and plovers from FUV and FAR largely resembled mainland plovers but showed signs of incipient genetic differentiation.

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

DNA extraction and amplification of 21 autosomal and one Z-linked microsatellite markers followed methods described in detail by Küpper *et al.* (2007, 2009). Microsatellite genotypes and sampling locations have been deposited at data dryad accession no. doi:10.5061/dryad.rr0tb. For mtDNA analyses, we used partial control region sequences described by Rheindt *et al.* (2011) and amplified partial fragments of the D-loop of the control region for samples of ten additional populations using the primers SNPL90 and TS778H (Wenink *et al.* 1994; Funk *et al.* 2007) using 20-μL polymerase chain reactions (PCRs). PCRs contained ~20 ng of DNA and 0.5 units of Taq DNA polymerase (Bioline) in the manufacturer's buffer with a concentration of 1.0 μM of each primer, 2.0 μM $MgCl_2$ and 0.20 mM of each dNTP. PCRs were carried out on a thermal cycler (MJ Research model PTC DNA engine) using the following program: one cycle of 3 min at 94 °C followed by 35 cycles of 94 °C for 30 s, annealing temperature of 55 °C for 30 s, 72 °C for 30 s, and a final extension cycle of 10 min at 72 °C. To check for amplification success, we visualized 5 μL of each PCR product on a 2% agarose gel stained with SYBRsafe (Invitrogen).

Products of successful PCRs were precipitated with ethanol and sequenced using Big Dye Terminator Cycle chemistry on ABI 3730 capillary DNA automated sequencers at the Natural Environmental Research Council Biomolecular Analysis Facility (NBAF) at the University of Edinburgh. In total, a 427-bp partial sequence of the D-loop for 245 Kentish plovers and three snowy plovers were available for the subsequent

analysis (Table 1). Sequences were aligned using the CLUSTALW algorithm implemented in CodonCode Aligner 2.0.0 beta 7.

Statistical analyses

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

We tested for genetic bottlenecks and demographic changes in two ways. First, for mtDNA, we calculated Tajima's D using the program DNASP (Librado & Rozas 2009). Negative Tajima's D values, if the marker is deemed neutral, may suggest a population expansion after a bottleneck, whereas positive values may suggest population size decrease. Second, for autosomal microsatellites, we used the coalescent method implemented in the program BOTTLENECK version 1.2.02 (Cornuet & Luikart 1996) and tested whether observed heterozygosity excess or deficiencies were indicative of a recent bottleneck or population expansion which would follow a bottleneck after colonization. As model for microsatellite evolution, we chose the two-phased model (TPM) and tested for statistical significance with Wilcoxon signed-rank tests.

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

Genetic differentiation among populations was estimated in three ways. First, we calculated Φ_{ST} values (mtDNA), F_{ST} and R_{ST} values (microsatellites) in ARLEQUIN. R_{ST} is expected to give more accurate differentiation estimates than traditional F_{ST} if the mutation process of the genetic markers resembles a stepwise process (Slatkin 1995; Balloux & Lugon-Moulin 2002). Pairwise differentiation coefficients were calculated between all 21 locations. Permutation tests with 1000 randomly generated $\Phi_{ST}/F_{ST}/R_{ST}$ values were used to test the probability of observed values arising by chance. Significance levels were adjusted using q-values to account for false discovery rates because of multiple testing (Storey 2002). Second, we used factorial correspondence analysis (FCA) to examine genetic differentiation of multilocus genotypes using the program GENETIX version 4.05 (Belkhir *et al.* 1996–2004). FCA is a multidimensional statistical method to visualize data that is superior to principal component analysis when discrete variables such as codominant microsatellite loci are involved. Third, we used two Bayesian clustering approaches to examine population differentiation with the autosomal markers. We used STRUCTURE version 2.1 (Pritchard *et al.* 2000) to estimate the number of clusters K in our data set and to assign individuals based on the admixture model with correlated allele frequencies to one or several clusters. With this approach, a proportion of each individual's genome is assigned to each cluster assuming gene flow among populations. We ran ten independent simulations with 500 000 generations following a burn-in of 250 000 for K ranging from 1 (no differentiation) to 21 (maximum divergence). We evaluated the assignment probabilities, log likelihood and ΔK (Evanno *et al.* 2005) to determine the optimal number of clusters. We then used the program TESS version 2.3.1 (Chen *et al.* 2007) to assign individuals to clusters and validate the number of clusters estimated with STRUCTURE. In TESS, we used the hierarchical mixture model where the prior distribution on cluster labels is determined by a hidden Gaussian random field (CAR model). This approach may provide lower error rates than other clustering methods when low levels of genetic structure are observed (Chen *et al.* 2007). For each K, we ran 50 iterations with 50 000 cycles after discarding a burn-in of 30 000 cycles and chose the best 10 runs (20%) according to the lowest Deviance Information Criterion (DIC) values. Average DIC values for

each K were plotted, and the most likely K was determined at the value where DIC values reached a plateau. For both STRUCTURE and TESS, we averaged the results of the best ten runs using CLUMPP version 1.1.2 (Jakobsson & Rosenberg 2007). Results of the processed runs were visualized with DISTRUCT version 1.1 (Rosenberg 2004).

We tested for isolation-by-distance in two ways. First, we used the Mantel's test implemented in ARLEQUIN to test for a general association of geographic distance with genetic differentiation using all sampled breeding population locations. Second, we carried out a linear regression to test whether the genetic differentiation of island populations was affected by their log-transformed distance to the mainland. We used the largest distance of open water that plovers originating from the mainland needed to cross to reach the island breeding locations ('ocean distance'), because we reasoned that plovers would use islands between the mainland and island breeding locations as stepping stones. As measure of genetic differentiation, we used Rousset's distance ($F_{ST}/(1-F_{ST})$ for microsatellites or $\Phi_{ST}/(1-\Phi_{ST})$ for mtDNA (Rousset 1997). Genetic differentiation was calculated for pairwise comparisons with each island population vs. the entire mainland population, and we estimated distances with the ruler function in Google Earth version 4.02 (Google 2007). Distances were $\log_{10}$ transformed before the analyses.

To estimate number of migrants ($4N_em$ for microsatellites and $2Ne_fm$ for mtDNA) and the Watterson estimators Θ, we used the coalescent approach implemented in MIGRATE version 3.2.6. We estimated $4N_em/2Ne_fm$ between, and Θ within all population clusters previously identified through STRUCTURE and TESS. After an initial burn-in of 25 000 000/100 000 (mtDNA/autosomal microsatellites), a long chain of 50 000 000/1 000 000 trees was sampled of which 50 000/2000 trees were recorded. Four-chain heating was used with temperatures set to 1, 1.2, 3 and 6 to improve tree space sampling. Each run was replicated five times, the Bayesian estimates of the previous run were used as initial estimates of these parameters for the subsequent run, and the values of the last chain were recorded. Two independent runs were carried out to confirm that final chains converged at highly similar estimates for modes and 95% confidence intervals, and we report the mean values of the two analyses.

We tested for sex-biased dispersal by comparing genetic estimates of migration and genetic differentiation of biparentally and uniparentally inherited markers in three ways. First, following Wright *et al.* (2005), we compared migration rates of mtDNA and autosomal microsatellites calculated in MIGRATE. The effective population size (N_e) of maternally inherited mtDNA is only 1/4 of biparentally inherited nuclear markers (Avise 2004). If the adult sex ratio of a population is 1:1, $4N_em$ estimates of nuclear markers divided by four can be compared with $2N_{ef}m$ rates of mtDNA. Differences are attributed to sex-biased dispersal. Second, we compared pairwise F_{ST} values derived from the Z-linked microsatellite marker with the values of the 21 autosomal microsatellites for all locations where we sampled at least two males (n = 20, PST was excluded). We only included genotypes of males for the calculation of the coefficient of genetic differentiation derived from the Z-linked marker, because females have only a single copy of the Z-chromosome. We used a Wilcoxon signed-rank test to examine statistical significance of autosomal and Z-chromosomal F_{ST} differences. No difference in genetic differentiation between Z and autosomal markers would suggest lack of sex-biased gene flow. Stronger differentiation at the Z marker than the autosomal markers indicates female-biased gene flow, whereas lower differentiation suggests male-biased gene flow. Third, we used a randomization method implemented in FSTAT version 2.9.3 to test whether pairwise F_{ST}, F_{IS} and relatedness differ between sexes (Goudet *et al.* 2002). The rationale for this test is that genetic differentiation and relatedness will differ between sexes if one sex largely stays at the natal site whereas the other sex disperses. For this approach, the difference between the genetic indices of differentiation or relatedness of adults from both sexes are calculated, and then, the sex is randomly assigned to each multilocus genotype of the original population sample keeping the original sex ratio intact. As the analysis is sensitive to sample sizes and power decreases with small sample sizes, we used the clusters previously identified with the Bayesian analyses to define populations and repeated this procedure 1000 times to examine statistical significance (Goudet *et al.* 2002).

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

Results

Genetic diversity and tests for bottlenecks

The 427-bp fragment of the Kentish plover control region contained 34 (8.0%) polymorphic sites, and no indels were present. Among 237 plovers sampled at breeding locations, we found 51 haplotypes (54 haplotypes among 245 samples from all 21 locations). Thirty-three haplotypes were exclusive to plovers from mainland breeding locations, ten haplotypes were

exclusively found in plovers from island breeding sites, and only eight haplotypes were shared between island and mainland breeding locations. However, the shared haplotypes included the three most frequently observed haplotypes and accounted for more than 50% of the haplotypes observed in both groups (islands: 56.5% and mainland: 63.2%).

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

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

Phylogenetic analyses

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

Genetic differentiation

Pairwise comparisons for mainland–mainland breeding sites revealed very low (or complete lack of) genetic differentiation across autosomal and sex-specific markers. R_{ST} values for microsatellite data and the majority of F_{ST} and Φ_{ST} values were low and nonsignificant for mainland–mainland comparisons (Tables S1 and S2, Supporting information). Only mitochondrial Φ_{ST} values between one of the locations from the centre of the continental distribution (ALW) and the three Iberian locations (SAM, FDP and DON) were significant, but the Φ_{ST} values were low and we interpret these results rather as stochastic effects of a single marker than biologically meaningful. None of the F_{ST} values calculated from the Z-linked marker were significant, but 13 of the 55 pairwise F_{ST} values for autosomal markers for mainland comparisons were. However, no autosomal F_{ST} value was larger than 0.03. Φ_{ST} values ranged from −0.05 to 0.14 (mean = 0.02, SE = 0.008), F_{ST} ranged from

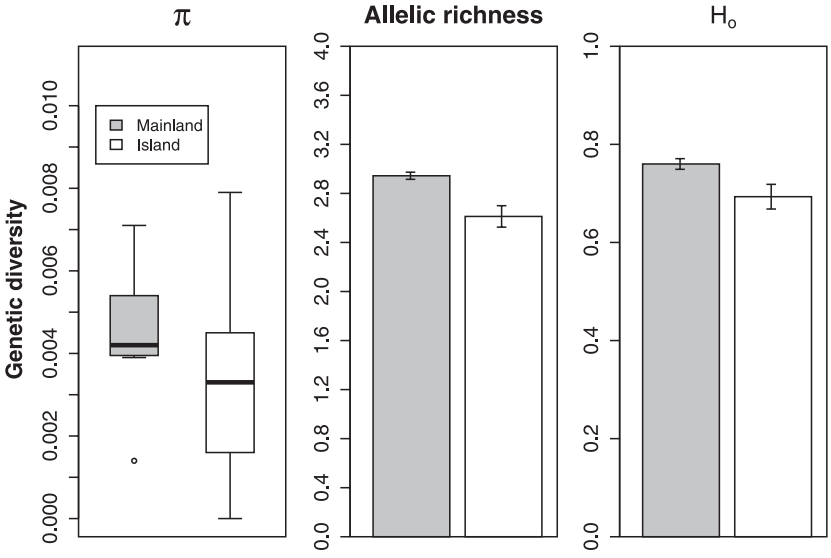

Fig. 2 Genetic diversity of nine island and eleven mainland breeding locations of Kentish plovers. There was no significant difference in mitochondrial sequence diversity π, but mainland breeding locations harboured higher nuclear genetic diversity (allelic richness and observed heterozygosity H_o) than island breeding locations based on 21 autosomal microsatellite markers. Median given for π, mean ± standard error given for allelic richness and H_o.

Table 2 Genetic diversity of 20 Kentish plover breeding locations at a 427-bp fragment of the mitochondrial control region and 21 autosomal microsatellite markers

	mtDNA				Microsatellites				
Population	n_{HT}	h	π	D_T	A	A_{rich}	H_o	H_e	P_{TPM}
Island									
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
Mainland									
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

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

−0.01 to 0.03 (mean = 0.01, SE = 0.002) and R_{ST} ranged from −0.02 to 0.04 (mean = 0.01, SE = 0.002) for mainland sites for which at least 10 individuals were sampled. For breeding sites that were separated by open ocean and for which at least ten individuals were sampled, most Φ_{ST}, F_{ST} and R_{ST} comparisons were highly significant. Φ_{ST} values ranged from −0.01 to 0.58 (mean = 0.22, SE = 0.019), F_{ST} values ranged from 0.02 to 0.17 (mean = 0.07, SE = 0.001) and R_{ST} ranged from 0 to 0.22 (mean = 0.08, SE = 0.006).

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

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

The two Bayesian analyses for cluster assignment suggested K = 5 as the most likely number of population clusters. Both analyses consistently flagged three separate clusters (Fig. 1): the Azores (STM), Cape Verde (CVB and CVM) and East Asian Islands (TWB, OKN and JAP). The wintering population (TWW) was intermediate between the Eastern Asian cluster and continental Eurasian Kentish plovers; a number of individuals had largely continental genotypes, suggesting

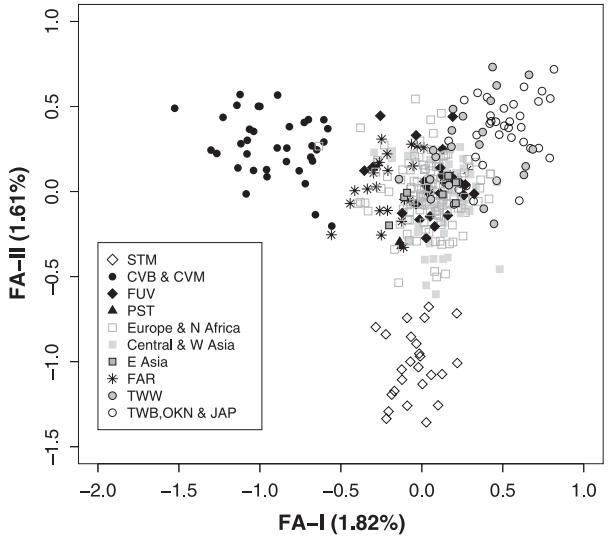

Fig. 3 Genetic differentiation of Kentish plover populations visualized with a factorial correspondence analysis. Island populations are presented with open or filled black symbols. Grey squares refer to plovers sampled during the breeding season at eleven mainland sites. Europe and N Africa include samples from SAM, DON, FDP, GHR, BLK and KUJ, Central and W Asia includes samples from TUZ, ALW and ELT, and E Asia includes samples from XIN and BOH.

that Kentish plovers from the mainland overwinter in Taiwan. There was disagreement about assignment to the remaining clusters between TESS and STRUCTURE. Results of STRUCTURE suggested two additional clusters: one for breeders from the Canary Islands (FUV) and one for the breeders from Farasan Islands (FAR) with the genomes of mainland Kentish plovers split about equally between these two clusters (Fig. 1). The genotypes of the two samples from PST were split between the East Asian and the FUV cluster. Results of TESS suggested one cluster for the mainland Kentish plovers and assigned the majority of the genotypes of the FUV, PST and FAR plovers to this cluster. However, a significant portion of the genomes (0.19 and 0.12, respectively) of the FUV and FAR plovers were attributed to a joint fifth cluster. For FUV and FAR plovers, other significant portions of the genomes were assigned to the CVB/CVM and STM clusters. In TESS, runs with higher K values assigned these parts of the FUV and FAR plover genomes to different clusters although the largest part of their genomes was still assigned to the mainland cluster.

Migration

Because of the uncertain assignment of FAR and FUV breeders, we calculated migration rates assuming six genetic clusters: (i) STM, (ii) CVB and CVM, (iii) FUV, (iv) mainland, (v) FAR and (vi) TWB, OKN and JAP. The two samples of breeders from PST were excluded from the migration analysis. Results of the two independent runs were consistent and very similar, indicating that the runs had converged.

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

Sex-biased dispersal

Comparisons using biparentally and uniparentally inherited markers supported the hypothesis of moderately female-biased gene flow. After adjusting for different N_e (by dividing nuclear estimates by four under the assumption of equal sex ratios), modal values for total migration rates (immigration and emigration rates combined) were higher for mtDNA than for microsatellites

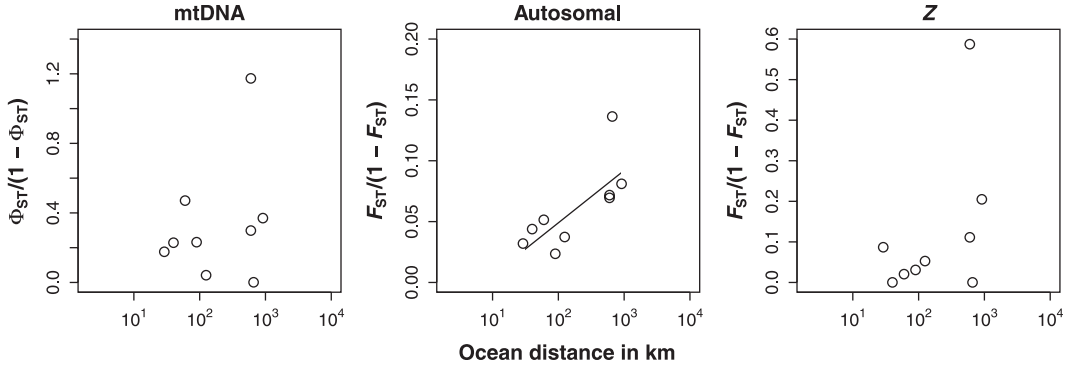

Fig. 4 Relationship between genetic differentiation and distance over open ocean of nine island locations vs the mainland for mitochondrial DNA, autosomal microsatellites and a Z-chromosomal microsatellite marker. Only autosomal microsatellites showed a significant linear relationship with distance.

Table 3 Estimates of Θ and *Nm* for genetic clusters of Kentish plovers estimated from 21 autosomal microsatellite markers and a 427-bp fragment of the mitochondrial D-loop using MIGRATE

Population	Θ	Cape Verde →	Santa Maria →	Fuerteventura →	Mainland →	Farasan Islands→	East Asian Islands →
MtDNA							
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)	
Microsatellites							
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)	2.5 (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)	

Owing to differences in the mode of inheritance, Θ and *Nm* values estimated from microsatellites were divided by four to make them comparable with corresponding values from mitochondrial DNA under the assumption of equal sex ratios. Migration rates were averaged over two converged independent runs with five replicates. Modal values with 95% confidence limits in parentheses are given.

(Table 3). Modal values for *Nm* from the islands to the mainland were on average two to four times higher for mtDNA, than modal values estimated from microsatellite markers although the *Nm* estimates from mtDNA showed large confidence limits. By contrast, *Nm* estimates were lower for mtDNA markers than microsatellite markers for gene flow from mainland to island clusters across all comparisons.

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

Discussion

Our results demonstrate unusually high gene flow across large geographic distances in a terrestrial bird species using mtDNA, autosomal and Z-linked microsatellites. Bayesian analyses of mtDNA and autosomal microsatellite loci show that mainland Kentish plovers are largely genetically undifferentiated across continental Eurasia and Africa. The genetic pattern of continental sampling locations that were separated by up to 10 000 km resembled the pattern in a single panmictic population. This lack of genetic structure cannot be explained by homoplasy of microsatellite markers or by low power of the applied marker set because we detected genetic differentiation of ocean island populations, and the panmixia pattern derived from mtDNA was consistent with the pattern observed at microsatellites. Island populations were moderately differentiated from the mainland populations and genetic differentiation increased with distance of the islands from mainland.

When analysing patterns of genetic differentiation, it is important to disentangle current gene flow from demographic processes that occurred in the population history (Avise 1994). Low genetic structure and sharing of haplotypes are seen in many species that have undergone a bottleneck and shifted their geographic distributions in response to climate oscillations such as the last glacial maximum (e.g. Wenink *et al.* 1994; Hewitt 2000). However, we argue that it is unlikely that the last glacial maximum has caused a profound shift of the Kentish plover distribution. First, in contrast to inhabitants of higher latitudes, most of the present distribution of Kentish plovers was not covered by the ice sheet during the last glacial maximum. The centre of the current distribution in Southern Europe, North Africa and Asia provided sufficient suitable habitats to maintain a substantial population (Cramp & Simmons 1983; Harrison & Prentice 2003). Second, we did not detect any

evidence for population bottlenecks or expansions at mtDNA or microsatellite markers for the continental population. Furthermore, the observed lack of an isolation-by-distance pattern supports the view that lack of structure is caused by high contemporary gene flow.

Lack of genetic structure across large geographic distances is rare among terrestrial animals and has only been described in a handful of insects and birds (Estoup *et al.* 1996; Beverdige & Simmons 2006; Funk *et al.* 2007; Reudink *et al.* 2011; Verkuil *et al.* 2012). By contrast, most other terrestrial species show at least modest genetic structure (Avise 2000). Based on the breeding ecology, we offer two explanations for the high gene flow. First, Kentish plovers often breed in temporarily available habitats such as salt marshes, alkaline lakes and fish ponds, and the long breeding season (which lasts up to 5 months) provides opportunities for several successful breeding attempts per year (Székely & Lessells 1993; Kosztolányi *et al.* 2009). Local breeding locations at temporal salt lakes are often unstable and only suitable for a fraction of the available breeding time promoting mobility of the breeders. Unpredictable and unstable habitats have been proposed to explain panmixia in Dawson's burrowing bees *Amegilla dawsoni* (Beverdige & Simmons 2006). Second, resighting and genetic data suggest high breeding dispersal particularly by females. During the reproductive season, Kentish plover females can breed at sites hundreds of kilometres apart, which will prevent breeding locations from differentiation (Székely & Lessells 1993).

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

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

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

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

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

female-biased gene flow as the most likely explanation for higher genetic differentiation of Z-chromosomal markers than autosomal markers.

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

It is also possible that the sex-biased gene flow is reduced by male natal dispersal. Higher natal dispersal by males has been reported in other polyandrous shorebirds (Clarke *et al.* 1997). We can only indirectly test natal dispersal using recruitment data because ringing recoveries of Kentish plover juveniles are scarce. Recruitment in two Kentish plover populations that were studied over a period of five or more years showed no sex bias: at FDP, a total of 16 males and 17 females that were ringed at hatching were recruited subsequently (Amat *et al.* 2001), and similarly, at TUZ, 32 male and 29 female recruits were caught over five field seasons (T Székely, A Kosztolányi, C Küpper, unpublished). Based on the observed strong adult male bias in polyandrous populations, the number of male recruits is surprisingly low and concordant with male-biased natal dispersal. If male-biased natal dispersal is a general distribution-wide behaviour, the observed female-biased gene flow would indicate contrasting magnitudes in dispersal at different life history stages with female breeding dispersal being much stronger than male natal dispersal. Alternatively, differences in reproductive success between male and female dispersers could lead to female-biased gene flow. In this case, we predict that dispersing males have lower reproductive success at their new locations than dispersing females.

Phylogeographic studies of *Charadrius* species seem to support a role of mating systems on population genetic structure. The closely related snowy plover shares many breeding biology characteristics with the Kentish plover such as multiple clutches, polygamy and nesting in unstable habitats (e.g. Warriner *et al.* 1986; Page *et al.* 1995). A number of snowy plover populations are well monitored, and a wealth of resighting data has been accumulated over the last decades. Both snowy plover males and females are highly site faithful, and more than 95% of male and female chicks return to breed at their natal sites in subsequent years (Stenzel *et al.* 2011). During the breeding season, snowy plovers are mobile and may breed at several locations up to 660 km (females) and 840 km (males) apart (Stenzel *et al.* 1994). Consistently, snowy plovers do not exhibit genetic structure across their North American continental range (Funk *et al.* 2007). Lack of genetic structure was also found in another plover species with a multiple clutch system, the mountain plover *C. montanus* (Oyler-McCance *et al.* 2008). In contrast, moderate population structure has been observed on a relatively small spatial scale in the monogamous piping plover *C. melodus* (Miller *et al.* 2010). Additional genetic studies of monogamous and low latitude breeders in this genus are needed to examine the association between mating systems and population genetics.

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

Despite the overall female-biased gene flow, we observed an interesting switch of sex-biased gene flow. Low mean values of mtDNA and higher mean values for microsatellites suggested male-biased gene flow from the mainland to the islands, whereas strongly female-biased gene flow was observed from the islands to the mainland (Table 3). This apparent difference in sex-biased gene flow could have two explanations. First, mainland and island plovers may differ in their dispersal behaviour or capabilities with island females and mainland males dispersing further and the

opposite sex dispersing less. Sex differences in migratory behaviour are known from other shorebird species with males and females wintering at different locations (Gill *et al.* 1995; Nebel *et al.* 2002) although it is not known whether these sex differences are population specific. However, we think that in Kentish plovers, this is unlikely because mainland females but not males were observed at distant breeding sites (Székely & Lessells 1993). Second, the apparent male bias could be an artefact because for the mainland to island comparisons, the confidence intervals for mtDNA were large and exceeded those of the microsatellites. Further studies are needed to test whether the apparent asymmetrical sex-biased gene flow has a biological meaning.

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

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

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

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C.K., S.V.E., T.B. and T.S. initiated the study. C.K., A.K., M.A. R., P.H., A.A.T., J.A.A., A.R., M.A., H.H., A.I., J.C. and T.S. collected samples at different geographic locations. C.K. analysed the genetic data. C.K., S.V.E., A.K. and T.S. interpreted the results. C.K. drafted the manuscript. All authors contributed to write up and approved the final version of the manuscript.

Data accessibility

mtDNA sequences: deposited in European Molecular Biology Laboratory database under accession nos AM941499–AM941501, AM941516–AM941551, FR822851–FR822903, FR822935–FR822941, FR822957–FR822981 and HE603647–HE603792. microsatellite genotypes and sampling locations: deposited in data dryad database doi:10.5061/dryad.rr0tb.

Supporting information

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

Table S1 Pairwise F_{ST} (above diagonal) and R_{ST} values (below diagonal) for 20 breeding locations and one wintering location of Kentish plover based on 21 autosomal microsatellites.

Table S2 Pairwise Φ_{ST} values based on a mitochondrial marker (above diagonal) and pairwise F_{ST} values based on a Z-linked microsatellite marker (below diagonal, calculated with males only) for 20 breeding locations and one wintering location of Kentish plover.

Fig. S1 Bayesian phylogeny based on a 427 bp mitochondrial DNA control region fragment of 245 Kentish plovers with three snowy plovers as outgroup (indicated by asterisk).