High fidelity: extra-pair fertilisations in eight *Charadrius* plover species are not associated with parental relatedness or social mating system

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Extra-pair paternity is a common reproductive strategy in many bird species. However, it remains unclear why extra-pair paternity occurs and why it varies among species and populations. Plovers (*Charadrius* spp.) exhibit considerable variation in reproductive behaviour and ecology, making them excellent models to investigate the evolution of social and genetic mating systems. We investigated inter- and intra-specific patterns of extra-pair parentage and evaluated three major hypotheses explaining extra-pair paternity using a comparative approach based on the microsatellite genotypes of 2049 individuals from 510 plover families sampled from twelve populations that constituted eight species. Extra-pair paternity rates were very low (0 to 4.1% of chicks per population). No evidence was found in support of the sexual conflict or genetic compatibility hypotheses, and there was no seasonal pattern of extra-pair paternity (EPP). The low prevalence of EPP is consistent with a number of alternative hypotheses, including the parental investment hypothesis, which suggests that high contribution to care by males restricts female plovers from engaging in extra-pair copulations. Further studies are needed to critically test the importance of this hypothesis for mate choice in plovers.

Social and genetic mating systems are often discordant, particularly in birds (Griffith et al. 2002, Matysioková and Remeš 2013). Although 90% of bird species are socially monogamous (Lack 1968), a large proportion of species are not genetically monogamous (Griffith et al. 2002, Westneat and Stewart 2003). Despite being the focus of behavioural research for almost three decades, the reasons why females engage in extra-pair copulations (EPCs) remain unclear (Burke and Bruford 1987, Birkhead et al. 1990, Jennions and

Petrie 2000, Griffith et al. 2002, Westneat and Stewart 2003, Akçay and Roughgarden 2007, Schmoll 2011, Forstmeier et al. 2014). Although a direct increase in fecundity is an immediate and obvious potential benefit to males (Petrie and Kempenaers 1998), it is still uncertain whether EPCs are an adaptive strategy of females or whether they could be the result of coercive strategies by males (Westneat and Stewart 2003, Griffith 2007, Adler 2010, Hsu et al. 2014).

Multiple factors may have influenced the evolution of extra-pair paternity (EPP) and the frequency at which EPP occurs within a given population. The degree of EPP varies greatly, both intra- and inter-specifically (Petrie and Kempenaers 1998, Arnold and Owens 2002). In order for EPCs to be adaptive for females, the benefits of engaging in EPCs must

outweigh the potential costs in terms of, for example, increased exposure to disease, loss of paternal care from the social mate and increased predation risk (Westneat and Stewart 2003). It has been suggested that females gain either direct or indirect benefits from engaging in EPCs. Direct benefits may include increased access to resources in return for engaging in sexual activity with a male (Gray 1997a, Petrie and Kempenaers 1998). Alternatively, EPCs could help to ensure fertility if the social partner for some reason has low fertility (Sheldon 1994, Gray 1997b). Other hypotheses suggest that females may gain indirect benefits, with offspring inheriting higher quality, 'good genes' or more compatible genes, which confer heterozygote advantages for the offspring, although evidence to support this hypothesis is mixed (Petrie and Kempenaers 1998, Tregenza and Wedell 2000, Akçay and Roughgarden 2007, Schmoll 2011, Hsu et al. 2014). The genetic compatibility hypothesis suggests that extra-pair paternity occurs to reduce the deleterious effects of inbreeding (Kempenaers et al. 1999, Tregenza and Wedell 2000, Blomqvist et al. 2002a, Thuman and Griffith 2005). This hypothesis assumes that EPC-seeking females are constrained in their choice of social mates and therefore sometimes pair with a genetically suboptimal mate. Individuals paired with genetically similar mates should therefore engage in more EPC and experience higher frequencies of EPP than less related pairs. Until recently, overall support for this hypothesis was considered to be weak (Akçay and Roughgarden 2007). However, a recent meta-analysis confirmed a significant positive relationship between EPP and pair relatedness (Arct et al. 2015). This inconsistency could reflect the incomparability of these studies due to methodological differences. Alternatively, it could be exposing true differences among genera, species or populations. Multi-species studies, where both field and laboratory work have been conducted following consistent protocols, eliminate such methodological heterogeneity and permit more reliable and robust tests of this and other hypotheses proposed to explain EPPs.

Another possible driver of EPP within a species or population could be sexual conflict (Westneat and Stewart 2003, Székely et al. 2007). Sexual conflict, the divergent evolutionary interests of males and females over reproduction (Arnqvist and Rowe 2005, Rice and Gavrilets 2014), may increase rates of EPP for two reasons. First, females may seek out copulations with extra-pair males if this increases their reproductive success even though these EPCs reduce the reproductive success of their social mate (Westneat and Stewart 2003, Székely et al. 2007). Interactions between the female, her social mate and other members of the population can lead to complex dynamics, since the social mate may retaliate and subsequently reduce his care (Chaine et al. 2014). Second, EPP can lead to brood desertion whereby one, or both, parents abandon their young and seek out new mates to increase their reproductive success (Székely 2014). Brood desertion often reduces the survival prospects of the young, thus the deserting parent gains increased reproductive success by pairing with a new mate, whereas the abandoned mate bears the full cost of raising the brood alone that may lower their expected survival until future breeding (Szentirmai et al. 2007). Sexual conflict theory therefore predicts that extra-pair young (EPY) will be more frequent in socially polygamous species, in part due to rapid mate-changes, compared to those that are socially monogamous and experience lower levels of conflict.

Additionally, the occurrence of EPP within a population sometimes follows a seasonal pattern (Dale et al. 1999, Küpper et al. 2004). One explanation is that the availability of high quality mates late in the breeding season is limited for females since most high quality males will already be paired up, meaning that females often end up pairing with a low quality mate. At the same time chick mortality often increases later in the season as environmental conditions deteriorate and competition between families increases (Székely and Cuthill 1999, Székely et al. 1999, Küpper et al. 2004, Kosztolányi et al. 2006, 2009). Females might then be more likely to engage in extra-pair copulations to obtain genetic benefits for their offspring and thereby increase offspring survival. Alternatively, higher rates of EPP in later clutches could be a result of sperm storage in sequentially polyandrous females (Dale et al. 1999, Oring et al. 1992, Küpper et al. 2004).

The genus *Charadrius* consists of 30 species of plover belonging to the order Charadriiformes, which breed on every continent except Antarctica (dos Remedios et al. 2015a). The social mating systems and parental care strategies of plovers vary greatly (Székely et al. 2006, 2007). Breeding systems vary from biparental care and monogamy to uniparental care and polygamy – where one parent deserts the brood and remates soon after hatching (Székely and Cuthill 1999, Kosztolányi et al. 2006). This diversity in mating systems and ecology within a single genus make plovers excellent study species in which to investigate the evolution of breeding systems (Vincze et al. 2013). Despite good knowledge of social mating systems in plovers, little is known about their genetic mating systems (Küpper et al. 2004).

Here, we carried out parentage assignment based on microsatellite genotyping of 510 families and 1071 offspring sampled from twelve populations of eight species to investigate the degree of concordance between plover social and genetic mating systems. Specifically, we tested whether the frequency of EPP was related to the social mating system as predicted by sexual conflict theory. We predicted that socially polygamous populations would have higher frequencies of EPP than more cooperative, socially monogamous populations. Secondly, we tested the genetic compatibility hypothesis using our multi-population data set. Plovers are appropriate models for investigating the genetic compatibility hypothesis because previous work suggested that this may be an important driver of EPP frequency in shorebirds (Blomqvist et al. 2002a, Thuman and Griffith 2005). We predicted that social pairs with higher genetic relatedness would be more likely to have EPY than more distantly related individuals. Finally, we tested whether the incidence of EPP was related to the timing of mating within the breeding season. We predicted that the frequency of EPP would be higher at the end of the breeding season to compensate for constrained mate choice and more challenging conditions for offspring survival.

Material and methods

Sample collection and DNA extraction

We collected samples from twelve populations constituting eight *Charadrius* species. These populations included five

populations of Kentish plover *C. alexandrinus* (KeP1-5 from the Azores, Cape Verde, Turkey, United Arab Emirates and Saudi Arabia) and one population each of the two-banded plover *C. falklandicus* and rufous-chested dotterel *C. modestus* (TbP and RcD from Falklands Islands), white-fronted plover *C. marginatus*, Kittlitz's plover *C. pecuarius*, Madagascar plover *C. thoracicus* (WfP, KiP, and MaP from Madagascar), snowy plover *C. nivosus* (SnP, Mexico), and red-capped plover *C. ruficapillus* (RcP, south-eastern Australia). Extra-pair parentage of KeP3 was analysed previously by Blomqvist et al. (2002a) and Küpper et al. (2004) based on DNA fingerprinting. The plover populations vary both in breeding systems and parental care strategies (Table 1).

Details regarding fieldwork and specific conditions for each population are described elsewhere (see references in Table 1). In brief across all populations: we searched for nests on foot or by car according to standardised field methods outlined by Székely et al. (2008). Adults were caught on the nest during incubation or while tending newly-hatched chicks using funnel traps. The majority of chicks were caught shortly after hatching in or around (i.e. within 20 m of) the known nest scrape. 25–50 μl of blood was collected for DNA extractions from the brachial or tarsal vein in adults or tarsal vein in chicks. Blood samples were stored either in Queen's Lysis Buffer (Seutin et al. 1991) or absolute ethanol until extraction. Parents and chicks were colour ringed or flagged to allow assignment of social parents during subsequent encounters of families in the field. All samples were collected between 1998 and 2014 (Table 1).

We extracted DNA using the ammonium acetate precipitation method (Nicholls et al. 2000). DNA quality was evaluated by visualising DNA on a 0.8% agarose gel stained with SYBRsafe (Invitrogen, Carlsbad, California) or ethidium bromide, and we assessed the quantity of DNA using a NanodropND800 (Thermo Fisher Scientific).

Genotyping

We used microsatellite markers to obtain genetic profiles for each sampled bird and assign parentage. We combined fluorescently labelled primer pairs into multiplexes and typed 11–26 microsatellite markers per species (Supplementary material Appendix 1 Table A1) plus 1–3 sexing primers

(Griffiths et al. 1998, Küpper et al. 2007). Kentish plover multiplexes designed by Küpper et al. (2009) were used to genotype Kentish, white-fronted and red-capped plovers. For the other five species, we tested marker sets from related species and markers with proven utility in other bird species for cross-amplification (Primmer et al. 1995, Piertney et al. 2002, Funk et al. 2007, Küpper et al. 2007, 2008, Dawson et al. 2010, 2013). We ran 2 μl PCR reactions including 1 μl Qiagen multiplex master mix and 1 μl primer mix (primer concentration 0.2 μM) with 1–15 ng DNA. We undertook PCRs using a programme beginning with 15 min at 95°C followed by 35 cycles at 94°C for 30 s, T_a (multiplex specific annealing temperature; Supplementary material Appendix 1 Table A1) for 90 s, 72°C for 60 s and a final extension cycle of 60°C for 30 min, in a thermal cycler (MJ Research TET-RAD2 DNA Engine). Diluted PCR products were loaded onto an ABI 3730 DNA fragment analyser for visualisation. We assigned allele sizes to alleles using GeneMapper ver. 3.7 software (Applied Biosystems). Individuals that were typed at fewer than 80% of the markers were excluded from further analysis. We calculated the frequency of null alleles and a combined non-exclusion probability of all markers for each population using the software Cervus ver. 3.0.3 (Kalinowski et al. 2007).

Parentage analysis

The number of families studied per population varied from 10 to 100 (Table 2). Our parentage assignment procedure had two steps. Firstly, we conducted parentage assignment using the social parents as sole candidate parents in Cervus ver. 3.0.3 (Kalinowski et al. 2007). Cervus provides a measure for the parentage by assessing the parentage likelihood of the candidate parent for a given offspring and provides the confidence of the parentage assignment relative to the critical logarithm of the odds (LOD) or Delta scores, which are generated through simulation. Simulation parameters were set as follows: 10 000 cycles, 90% candidate parents sampled, 5% loci mistyped (null alleles or genotype mismatch) and population-specific numbers for loci and candidate parents. Although theory suggests that the parent and offspring should match genotypes completely, mismatches can occur due to mutations or

Table 1. Social mating system, study years, parental care strategy and population information for the 12 study populations.

Species	Population	Years	Parental care strategy	Reference
Polygamy				
Snowy plover	Mexico	2006–2010	Male only/biparental	Argüelles-Ticó 2011
Kittlitz's plover	Madagascar	2006–2013	Male only	Parra et al. 2014
Kentish plover	Turkey	1998–1999	Male only/biparental	Argüelles-Ticó 2011
Kentish plover	UAE	2005–2006	Uni/biparental care	Kosztolányi et al. 2009
Monogamy				
Kentish plover	Cape Verde	2007–2013	Biparental care	Argüelles-Ticó 2011
Kentish plover	Saudi Arabia	2011	Biparental care	AlRashidi et al. 2011a
White-fronted plover	Madagascar	2006–2013	Biparental/male only	Parra et al. 2014
Madagascar plover	Madagascar	2009–2013	Biparental care	Zefania et al. 2008
Unknown mating system				
Kentish plover	Azores	2009–2012	Likely biparental care	
Two-banded plover	Falklands Islands	2005–2008	Biparental care	St Clair et al. 2010a, Székely, unpubl. data
Rufous-chested dotterel	Falklands Islands	2005–2008	Biparental care	St Clair et al. 2010b, Székely, unpubl. data
Red-capped plover	Australia	2010–2014	Biparental care	Ekanayake et al. 2015

Table 2. The occurrence of extra-pair paternity (EPP), quasi-parasitism (QP) and intraspecific brood parasitism (IBP) across twelve plover populations. The strict dataset includes only families for which candidate parents were determined unambiguously in the field and therefore provides a minimal estimate of the occurrence of the alternative mating strategies. By contrast, the relaxed values provide initial parentage assessment including possible mis-assignments that may have been the result of wrongly assigned parentage in the field. The first line for each population provides the summary of broods, the second line in italicised font provides the summary of chicks.

Species	Population	Strict N broods *chicks*	EPP% (*n*)	QP% (*n*)	IBP% (*n*)	Relaxed N broods *chicks*	EPP% (*n*)	QP% (*n*)	IBP% (*n*)
Snowy plover	Mexico	93	0	0	0	100	0	0	0
		201				*225*			
Kittlitz's plover	Madagascar	15	0	0	0	31	6.3 (2)	3.1 (1)	6.3 (2)
		18				*44*	*4.3 (2)*	*2.2 (1)*	*4.3 (2)*
Kentish plover	Turkey	73	4.1 (3)	0	1.4 (1)	94	4.3 (4)	0	1.1 (1)
		184	*1.6 (3)*		*0.5 (1)*	*249*	*1.6 (4)*		*0.4 (1)*
Kentish plover	Cape Verde	63	1.6 (1)	0	3.2 (2)	90	2.2 (2)	0	3.3 (3)
		120	*0.8 (1)*		*1.7 (2)*	*174*	*1.7 (3)*		*1.7 (3)*
Kentish plover	UAE	28	0	0	0	48	4.2 (2)	0	2.1 (1)
		57				*101*	*2.0 (2)*		*2.0 (2)*
Kentish plover	Azores	0	–	–	–	19	0	0	0
						46			
Kentish plover	Saudi Arabia	12	0	0	0	27	3.7 (1)	0	0
		19				*54*	*1.9 (1)*		
Two-banded plover	Falklands Islands	6	0	0	0	10	0	0	0
		15				*26*			
Rufous-chested dotterel	Falklands Islands	8	0	0	0	12	0	0	0
		14				*19*			
White-fronted plover	Madagascar	10	0	0	0	29	0	3.45 (1)	3.45 (1)
		17				*49*		*4.08 (2)*	*2.04 (1)*
Madagascar plover	Madagascar	12	0	0	8.3 (1)	28	0	0	3.6 (1)
		20			*5.0 (1)*	*46*			*2.2 (1)*
Red-capped plover	Australia	4	0	0	0	22	9.1 (2)	13.6 (3)	4.5 (1)
		7				*38*	*7.9 (3)*	*10.5 (4)*	*2.6 (1)*

genotyping error (Hoffman and Amos 2005). Cervus therefore uses a probability-based approach to account for those mismatches, which occasionally can lead to unlikely results considering other sources of knowledge about the parents (Bouwman et al. 2006). Therefore, we reviewed the relationship between social parents and chicks and determined the number and nature of mismatches. We accepted social parents as genetic ones if they and their putative chicks had no more than one true mismatch and one null allele mismatch. We checked all incidences of two or more allele mismatches in detail. Mismatches caused by null alleles are a particular problem when using markers developed outside the study species, since the match between primer and target sequences may be poorer than in the species for which the marker was developed. Consequently, null allele frequencies tend to increase with increasing phylogenetic distance from the target species (Li et al. 2003), which could potentially lead to the false exclusion of true parents (Dakin and Avise 2004). On the other hand, dropping all markers affected by null alleles severely reduced exclusion probabilities in some populations. Therefore, we only dropped markers affected by null alleles for a given species until the combined non-exclusion probability score had dropped below 0.95 (Supplementary material Appendix 1 Table A2 for non-exclusion probabilities). In these cases, (i.e. rufous-chested dotterel, Kittlitz's, white-fronted, Madagascar and snowy plover), we only rejected parents if parent-offspring dyads showed at least three mismatches, including one 'true' mismatch that could not be explained by null alleles. Instances where both parents did not match the chick's genotype were classified as cases of intra-specific brood parasitism (IBP) and

cases where only the female was excluded were classified as quasi-parasitism (QP). IBP can occur when a female lays an egg in another pairs' nest, whereas QP occurs when a female lays an egg in the nest after copulating with the paired male first (Yom-Tov 2001, Griffith et al. 2004, Lyon and Eadie 2008, Berger et al. 2014).

Mismatches between parents and offspring can also result from incorrect assignments of parents during laboratory or fieldwork due to human errors. In families where chicks did not match the parents, all individuals were therefore independently re-extracted and re-genotyped to ensure errors during laboratory work were not responsible for the observed mismatches. For all mismatched families remaining in the dataset (n = 26), we also excluded other error sources, for example, by confirming the sex of putative parents using molecular markers (n = 7) to ensure that a male and a female were always assigned as social parents during fieldwork.

Potential sources of parental mis-assignments introduced during fieldwork by sampling the wrong precocial chicks are given in Table 3. Since we were primarily interested in the frequencies of EPY that resulted from alternative mating behaviour, we created two datasets: 1) a high-stringency dataset ('strict' dataset), which includes only broods with both parents sampled or identified during incubation before the last egg had hatched; and 2) a low-stringency data set ('relaxed' dataset), including additional families that originated from an undiscovered nest, or for which the chicks or parents were sampled after they had left the nest scrape. In addition, we also thoroughly checked records of field observations for all families where the social parents were not the genetic parents to identify and eliminate further potential sources of error.

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Table 3. Potential sources of error for parentage assignment in the field in precocial birds, identified and addressed by this study. The relaxed dataset included all sampled families.

Reason for mismatch	Potential conclusion	Treatment in this study
Brood mixing	Apparent case of IBP. May occur when offspring were first marked and sampled after they had left the nest scrape.	Excluded from strict dataset.
Parents not caught on nest during incubation	Apparent case of EPP, QP or IBP. Occasionally adult plovers that are not the true parents are caught with chicks and assigned as candidate parents in the field.	Excluded from strict dataset if no further family re-sightings with the candidate parents exist from later dates.
Other errors during parentage assignment in the field	Apparent case of EPP, QP or IBP.	Excluded from strict dataset if field notes during capture suggested that initial assignment was uncertain.

Social vs genetic mating systems

We tested whether the social breeding system was related to the degree of EPP using Fisher's exact test. For this analysis, populations were counted as either having an incidence of EPP/extra-pair fertilisation (EPF, where either the male or female were excluded as the genetic parent) or no EPP/EPF. Eight populations were included where information was available on both breeding system and parental care strategies. We defined a population's breeding system using a combination of their mating system and parental care strategy. Populations were classified as polygamous (snowy plover, Kittlitz's plover and Kentish plover from Turkey and United Arab Emirates) if they exhibit high levels of within season sequential polygamy ($>5\%$; Dunn et al. 2001, Eberhart-Phillips unpubl. data) and also uniparental care (Table 1). Monogamous populations (Madagascar plover, white-fronted plover and Kentish plover from Cape Verde and Saudi Arabia) were defined as having high levels monogamy within and among seasons (AlRashidi et al. 2011a, Eberhart-Phillips unpubl. data) and biparental care (Table 1). It is important to note however, that variation in mating and parental care strategy does occur within all populations.

Pair relatedness and temporal patterns

We used EPP/EPF presence or absence within a brood as the dependent variable and fitted population nested within species as a random factor in a series of generalized linear mixed models (GLMMs). EPF was only tested in the relaxed dataset, as there were no incidences of QP within the strict dataset, meaning that strict EPP rates equalled EPF rates. We calculated pair relatedness, i.e. the relatedness between the social father and mother, using Queller and Goodnight's r (Queller and Goodnight 1989) in the program SPAGeDi ver. 1.5 (Hardy and Vekemans 2002) to test whether parental relatedness predicted the occurrence of EPFs. To test for a temporal pattern of EPP we included the standardised hatching date as a main factor along with relatedness in the GLMM. For each population, we calculated standardised hatching date for each nest based on the available data by subtracting the mean and dividing by the standard deviation of each population. This made hatching dates comparable across the populations, as different populations have differently timed breeding seasons. We also tested the interaction between pair relatedness and standardised hatching date to examine potential temporal constraints on mate choice. These analyses were only conducted on populations and nests where hatching date information was available; Kentish plovers from the Azores, two-banded plovers and rufous-chested dotterels were excluded from both the strict and relaxed dataset analyses, whereas the red-capped plover was excluded from the strict dataset analysis only. We fitted a GLMM with a binomial error structure and a cloglog link function to the data. The cloglog link function allows for more asymmetry in the distribution than the standard logit link function. From saturated models that included all independent variables (relatedness and hatching date, and one two-way interaction), we sequentially removed non-significant interactions and variables until the minimal model was reached (Supplementary Material Appendix 1 Table A3). We tested the significance of the increase in deviance as a result of model simplification using chi-square tests.

For parents with multiple broods, we selected one randomly chosen record to avoid pseudoreplication (families excluded: relaxed dataset $n = 28$; strict dataset $n = 14$). For the correlates of EPFs, we report the results of the strict dataset plus those of the Kentish plover from the Azores for which we did not detect any EPFs (see Results). However, the trends were qualitatively the same for the relaxed dataset when only using populations with large sample sizes. We used R ver. 3.2.3 (R Core Team) for all statistical analyses.

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.k7797> (Maher et al. 2017).

Results

Parentage analysis and social vs genetic mating systems

A total of 1071 offspring and 978 adults constituting 510 families from twelve populations were genotyped using 11–26 microsatellite loci. The frequency of EPP was rare ($<5\%$) within each population. All offspring were included in the relaxed dataset and 718 (67%) offspring from 343 (67%) families were retained in the strict dataset, including families of Kentish plover from the Azores. Analysis of the strict dataset yielded EPP rate estimates between zero and 4.1% of broods (Fig. 1; Table 2). There was no incidence of QP
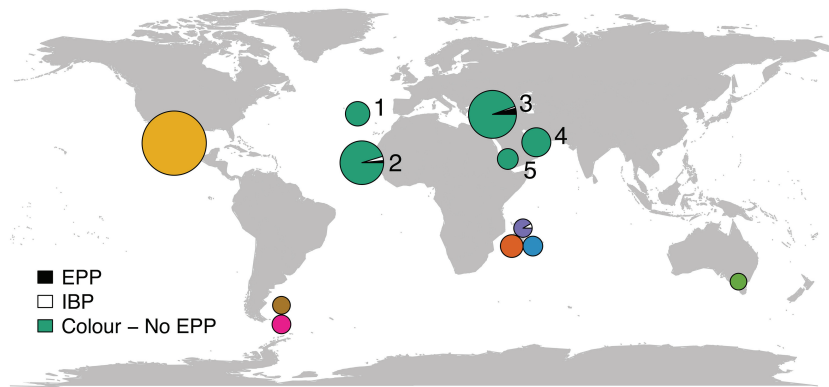
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Figure 1. Occurrence of extra-pair paternity (EPP) and intraspecific brood parasitism (IBP) in *Charadrius* plovers based on the strict dataset plus Kentish plover from the Azores (see Table 2 and 3). Snowy plover (yellow), two-banded plover (brown), rufous-chested dotterel (pink), Kentish plover (dark green: 1 = Azores; 2 = Cape Verde; 3 = Turkey; 4 = United Arab Emirates; 5 = Saudi Arabia), Madagascar plover (purple), Kittlitz's plover (orange), white-fronted plover (blue) and red-capped plover (light green). Sample sizes of broods are indicated by the diameter of population specific pie-charts.

(Fig. 1; strict dataset, Table 2). The rate of IBP was estimated at between zero and 8.3% of broods (Fig. 1; strict dataset, Table 2). Analysis of the relaxed datasets yielded somewhat higher EPP, IBP and QP estimates in most populations (relaxed dataset, Table 2). Monogamous and polygamous populations did not differ in their occurrence of EPFs or EPPs (Fisher exact test: p = 1).
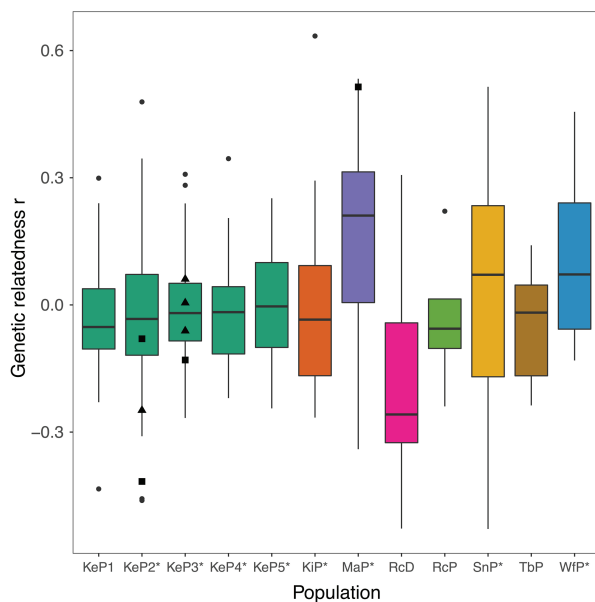
Figure 2. Relatedness (Queller and Goodnight's r) between social parents and the occurrence of extra-pair fertilisations across twelve plover populations based on the strict dataset plus Kentish plover from the Azores. The relatedness of pairs where extra-pair paternity occurred is represented by black triangles and where intraspecific brood parasitism occurred is represented with black squares. Black circles represent outliers. KeP1: Kentish plover, Azores, KeP2: Kentish plover, Cape Verde, KeP3: Kentish plover, Turkey, KeP4: Kentish plover, United Arab Emirates, KeP5: Kentish plover, Saudi Arabia, KiP: Kittlitz's plover, MaP: Madagascar plover, RcD: rufous-chested dotterel, RcP: red-capped plover, SnP: snowy plover, TbP: two-banded plover, WfP: white-fronted plover. Asterisks represent populations that were included in GLMMs carried out on the strict dataset.

Pair relatedness and temporal patterns

Pair relatedness of parents was low across all populations except for the Madagascar plover (Fig. 2). Pair relatedness and its interaction with standardised hatching date had no influence on the occurrence of EPPs and hence was not retained in the minimal model (GLMM; interaction: $\chi^2 = 0.0025$, df = 1, p = 0.96, pair relatedness: $\chi^2 = 0.33$, df = 1, p = 0.56, Fig. 2, Supplementary Material Appendix 1 Table A3). Interestingly, the single case of IBP that occurred in the Madagascar plover occurred in a family where both parents were very closely related ($r = 0.514$). For the strict dataset, we observed that although EPPs tended to be more frequent during the latter part of the breeding season (estimate = 0.99, ± 0.94 [SE], Z = 1.472, p = 0.14), time of the breeding season was not significant and therefore eliminated during model simplification to create the minimal model (GLMM: $\chi^2 = 3.81$, df = 1, p = 0.051, Supplementary material Appendix 1 Table A3).

Discussion

Social and genetic mating system of plover populations

Our results suggest that EPP is a relatively rare strategy with limited importance in plovers. Across all 12 populations we found low rates of EPF in both the strict and relaxed datasets. This is consistent with previous work on other *Charadrius* species where low rates of EPP were reported: ringed plover *C. hiaticula* (0% EPP, Wallander et al. 2001), semipalmated plover *C. semipalmatus* (4.2% EPP, Zharikov and Nol 2000) and Eurasian dotterel *C. morinellus* (9.1% EPP, Owens et al. 1995), suggesting this is a widespread trend among plover species. This contrasts with high frequencies of EPP found in other bird clades, with the highest rates found in the superb fairy-wren *Malurus cyaneus*, for example, where up to 76% of offspring are sired by an extra-pair male (Mulder et al. 1994).

One possibility is that low EPP rates could result from mate guarding and social constraints. Many plover species

only produce a single clutch of three or four eggs, suggesting that the cost of even a single EPC may be unusually high for the male (Wallander et al. 2001). Behaviours that reduce loss of paternity should therefore be under strong selection in males (Westneat and Stewart 2003, Thomas et al. 2007). Mate guarding occurs in some but not all plover species (Zharikov and Nol 2000, Wallander et al. 2001) and plover breeding habitat is often open with little cover (Muir and Colwell 2010) making it difficult for females to engage in EPCs without observation or disruption from the social mate (Delehanty et al. 1998, Zharikov and Nol 2000, Wallander et al. 2001, Küpper et al. 2004). Plovers might also employ paternity insurance strategies, such as high rates of within-pair copulations which act as paternity assurance for the social male (Emlen et al. 1998, Schamel et al. 2004). Other methods of paternity assurance could potentially also occur, such as in polyandrous wattled jacana *Jacana jacana*, where males have been observed discarding the first egg laid in the clutch except during the first breeding attempt of the season (Chen et al. 2008).

Alternatively, females might be constrained by their reliance on male cooperation during parental care, which might discourage them from seeking EPCs (Wallander et al. 2001, Blomqvist et al. 2002b, Arnqvist and Kirkpatrick 2005). Male parental care is important in many shorebird species and the costs of reduced parental care can be high when the remaining parent is unable to compensate fully (Székely and Williams 1995, Székely and Cuthill 1999, Blomqvist et al. 2002b, Thomas et al. 2007). Biparental care is particularly important for plovers breeding in harsh environments (Kosztolányi et al. 2009, AlRashidi et al. 2011b, Vincze et al. 2013). Therefore, females should be under selection to avoid EPCs if they lead to a reduction in care, desertion or divorce from their social mate. Although Kentish plover parents do not discriminate between unfamiliar eggs and chicks (Székely et al. 1994, Székely and Cuthill 2000), we cannot exclude the possibility that males can assess the certainty of their parentage and adjust their contribution to care accordingly (but see Alonzo 2010).

Alternatively, females might not necessarily benefit from engaging in EPCs. EPCs could be costly to the female in terms of increased exposure to sexually transmitted diseases or parasites, increased predation rates, time wastage or male retaliation (Sheldon 1993, Gowaty 1996, Arnqvist and Kirkpatrick 2005). If EPPs do not benefit females and are indeed costly, then there should be selection against females engaging in EPCs. Moreover, in polyandrous plovers such as snowy and Kentish plovers, females can mate sequentially with different males. In these species, males provide most of the brood care (Cruz-López et al. 2017) and are expected to ensure that they sire the offspring they care for.

Forced copulation seems an unlikely explanation for our results as there is little evidence for this in shorebirds. Females have been observed to resist extra-pair copulations and act aggressively to males other than their social mate (Lanctot et al. 2000). Plovers of our study populations have only moderate sexual size dimorphism (dos Remedios et al. 2015b), which would probably allow females to resist enforced copulations by males.

We found that the genetic mating system did not reflect the social breeding system in our study as the level of EPP was low in both monogamous and polyandrous plovers. Interestingly, EPP was also consistently low across a number of populations of the Kentish plover with contrasting social breeding systems. However, because of the generally low frequencies of EPP and the moderate sample sizes, our statistical tests may have had limited power to pick up subtle differences. Thomas et al. (2007) found that among socially monogamous shorebirds, those expressing higher levels of cooperation had lower levels of EPP. Extra-pair offspring are often more common in species experiencing higher levels of social conflict, such as polygynous and polyandrous species (Oring et al. 1992, Lanctot et al. 1997, Emlen et al. 1998, Lank et al. 2002, Székely et al. 2007, Thomas et al. 2007). Such an association was not evident in our analysis of twelve plover populations. Both monogamous and polygamous populations of Kentish plover had less than 5% EPP. Therefore, sexual conflict is unlikely to be responsible for the differences in EPP rates we found in plovers. A similar pattern of low EPP in polyandrous species has been observed in several other shorebird species (Owens et al. 1995, Delehanty et al. 1998, Dale et al. 1999, Schamel et al. 2004). This lack of association can perhaps be explained by the observation that polygamous plovers are typically sequentially polyandrous and exhibit partial biparental care, females assisting with incubation but not with brood care after hatching. The low frequency of EPP found in these polyandrous species may therefore reflect the importance of paternal care. This is supported by previous work which found relatively low rates of EPP in the Eurasian dotterel, a species that exhibits sex-role reversal (i.e. females are the more showy competitive sex and males provide nearly all of the parental care; Owens et al. 1995).

Pair relatedness

We found no evidence that parental relatedness was correlated with the likelihood of extra-pair young. As the relatedness of the parents was low across all populations except the Madagascar plover, inbreeding risk is probably low and thus EPFs may be unrelated to genetic factors. These results are not consistent with previous studies in shorebirds that found higher rates of EPP when parental relatedness was high (Blomqvist et al. 2002a, Küpper et al. 2004). We did not find this relationship across (nor within) plover populations despite using a relatively large number of reasonably polymorphic microsatellite markers (Supplementary material Appendix 1 Table A1). One of the reasons for this could be methodological differences, as DNA fingerprinting was used in many of the earlier studies. Microsatellite analysis is considered to be more accurate than DNA fingerprinting when judging genetic similarity between individuals (Reeve et al. 1992), which is reflected by the meta-analysis of Arct et al. (2015), who found significant positive effect sizes of EPP and relatedness only for microsatellites and not other markers. This is the opposite of our findings when comparing results between studies on the same species, as we found no effect of relatedness when microsatellites were used and previous studies were based on minisatellite probes (Blomqvist et al. 2002a, Küpper et al. 2004). Thus our work highlights

the importance of using consistent methodology, both in the field and laboratory with stringent filtering according to data quality, which allows for more reliable parentage inference.

Temporal patterns

There was a non-statistically significant trend for EPPs to be more frequent later in the breeding season. This trend is in line with previous findings in a single population of Kentish plovers (Küpper et al. 2004) and therefore consistent with the predictions of an adaptive strategy when females are paired with inferior mates. However, since the temporal trend was weak and EPPs in general were rare, further work will be required to test whether such a strategy could play a role more widely in plovers.

QP, IBP and EPP

Evidence for QP and IBP has been found in shorebirds and other bird species (Yom-Tov 2001, Blomqvist et al. 2002a, Arnold and Owens 2002, Griffith et al. 2004, Küpper et al. 2004, Krakauer 2008, Du and Lu 2010). IBP occurs when a female lays an egg in a conspecific pair's nest that she does not attend (Yom-Tov 2001, Berger et al. 2014). This can be detrimental to the fitness of the male and female recipients, as they are forced to provide care for the parasitic chick (Petrie and Møller 1991). QP occurs when a female engages in copulations with an already-paired male and then lays an egg in the paired female's nest, so that the social female incurs the fitness cost of raising the extra-pair offspring (Griffith et al. 2004, Lyon and Eadie 2008). It has been suggested that both IBP and QP occur when a female engages in EPCs with a male and then dumps an egg, sometimes in the same male's nest (Küpper et al. 2004). The rare occurrence of IBP and absence of QP within the strict dataset suggests that these are not major strategies in plovers. It is important to note that cases of EPP and QP were much more common in the relaxed dataset, although in general the trends we found were consistent between the two datasets. Differences in the frequency of EPP, QP and IBP between the relaxed and strict datasets could reflect inaccuracies in field assignment. This highlights the benefit of large datasets, as firm conclusions can still be drawn from a subset of the data, and also emphasises the need to have stringent field protocols. It is also important to recognise that the levels of EPP, QP and IBP reported are conservative estimates as the capacity to detect extra-pair offspring was limited in some species, such as snowy plover, rufous-chested dotterel and Madagascar plover, due to low exclusion probabilities (Supplementary material Appendix 1 Table A2).

In conclusion, there have been several hypotheses proposed to explain variation in levels of EPP in birds. Using 12 populations of plover and stringent data filtering, we found no significant association between social breeding system, pair relatedness and temporal patterns and EPP in plovers. Instead, EPP rates were consistently low across all studied populations. This is consistent with efficient mate guarding and/or a constraint on EPC engagement by females due to the substantial share of parental care that males provide. Further work is needed to critically test this hypothesis. It would be particularly interesting to test whether polygynous populations, in which females provide the majority of parental care, have higher levels of EPP.

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Supplementary material (Appendix JAV-01263 at <www.avianbiology.org/appendix/jav-01263>). Appendix 1.