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Abstract: Experimental assessment of mating opportunities in three shorebird species

Mating opportunities may differ between closely related species, although the evidence for such variation is scant. Here we compare remating opportunities and courtship behaviour between three shorebird species: the Kentish plover (*Charadrius alexandrinus*), the Kittlitz's plover (*C. pecuarius*) and the white-fronted plover (*C. marginatus*), using data and an experimental approach previously developed for the Kentish plover. By experimentally creating unmated males and females, we show that remating opportunities are different between closely related plover species (*Charadrius* spp): remating times were shorter for females than males in a Kentish plover population that exhibits a male-biased adult sex ratio, and where the majority of brood care after hatching is carried out by males. In contrast, remating times were male-biased in the uniparental Kittlitz's plover and unbiased in the biparental white-fronted plover. We also show that male Kentish plovers spend significantly more time on courtship than females, whereas courtship behaviour is not sex biased in the other two plover species. The mate-removal experiments also provided insights into pair bond stability. In the Kittlitz's plover, all 16 newly formed pairs remained together after the release of their former mates from captivity, whereas newly established pairs were replaced by their former mates upon release in 12 out of 12 white fronted plover pairs. Taken together, these results are important in highlighting interspecific variation in mating activities, and suggest that both operational sex ratio (OSR) and pair bond stability may differ between closely related species. These variations in turn, may influence mating systems and parental care.

Experimental assessment of mating opportunities in three shorebird species

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December 10, 2013

Dear Dr. Smiseth

Parra et al: Experimental assessment of mating opportunities in three shorebird species

Please find attached our revised manuscript for consideration as a research article in *Animal Behaviour*.

We much appreciate the constructive and detailed comments of the Editor before the paper will be publishable. We have carefully considered all comments and have revised the manuscript accordingly. Please find our detailed responses below. All changes have been highlighted in the uploaded revision.

Best regards,

Jorge E. Parra
PhD student

Editorial letter (original text italicised, and our responses are in plain font)

Formatting changes:

1. *Put keywords in alphabetical order.*

This has been modified in the revised manuscript, (lines 22 – 23)

2. *Use APA style for citations and references.*

This has been modified in the revised manuscript.

3. *For 'unpublished data' references include authors' initials and also a date if available.*

Done, thank you (see lines 97 - 98, 101 and 129)

4. *Tables should have a short one-sentence title above the table. Put other information below the table.*

Done, (see lines 545, 554 – 555 and 561)

5. *Tables 2, 3. Remove the internal horizontal lines.*

Done, (see tables 2 and 3)

6. *Make the supplementary table into an appendix table and include it with the other tables in the manuscript.*

Thanks, this has been changed accordingly (see lines 566 – 571)

Editor's additional comments:

(1) *Please delete the reference to Liker et al. unpublished data. This reference is not useful to the reader as the data are unpublished and it does not appear to be required given that you cite published work by the same author. If the reference is required, I would suggest that you cite it as personal communication.*

Thanks, this has been deleted accordingly.

(2) *Line 90: Delete the word 'the' to read 'White-fronted plovers and Kittlitz's plovers .'*

Done, the word was deleted accordingly (see line 94)

(3) *Line 134: Change 'arthropods' to 'invertebrates' as earthworms are annelids (and not arthropods).*

Done, thank you (see line 138)

(4) *Lines 149, 150 and elsewhere: Change 'mins' into 'min'.*

Done, thank you (see lines 153 and 155)

(5) *Line 158: Change semicolon into period.*

Done, thank you (see line 162)

(6) *Line 207: Change 'minutes' into 'min'.*

Done, thank you (see line 214)

(7) Line 237: Consider changing the subheading 'Between Species Prediction' to 'Between Species Comparison'.

Thanks, this has been changed accordingly; (see lines 78 and 248)

(8) Line 247: Please name the test statistic of the Gehan-Wilcoxon test and report the degrees of freedom.

Done, thank you (see line 258)

(9) Line 249: Consider changing the subheading 'Between Sexes Prediction' to 'Between Sexes Comparison'.

Thanks, this has been changed accordingly; (see lines 82 and 260)

(10) Lines 257 and 273-275: Please name the test statistic and report the degrees of freedom for the LSD tests.

Thanks, the test statistic and degrees of freedom have now been added (see lines 265, 268, 284 – 287)

In addition, Fisher's least significant difference test (LSD test) has now been explained in the manuscript as follows (see lines 202 – 204):

“Pairwise multiple comparisons were performed to compare mean differences of mating time between males and females of the three species using Fisher's least significant difference tests (LSD test)”

See also lines 222 – 223:

“Pairwise multiple comparisons were performed to compare mean differences of courtship behaviour between males and females in the three species of plovers using LSD tests”

(11) Line 266: Consider changing the subheading 'Courtship Prediction' to 'Courtship Behaviour'.

Thanks, this has been changed accordingly; (see lines 84 and 277)

(12) Lines 284-285: Please name the test statistic and report the degrees of freedom of the Fisher's exact test

Fisher's exact test does not have degrees of freedom. This test has been explained in the manuscript (see lines 224 – 225):

“Finally, pair bond stability was analysed comparing the frequencies of mate replacement between white-fronted plover and Kittlitz's plover with Fisher's exact test”

In addition (see lines 296 – 297):

“Fisher's exact test: mate replacement in white-fronted plover =12, N = 12; mate replacement in Kittlitz's plover = 0, N = 16; P < 0.001”

(13) Lines 291-292: Change '. ecological explanations are unlikely to explain .' to something like '. ecological factors are unlikely to explain .'.

Thanks, this has been changed accordingly; (see line 304)

(14) Lines 334-335: Please reword this sentence as it is unclear whether you mean to say that male and female plovers behave similarly despite having conventional sex roles or whether you mean to

say that the finding that male and female plovers behave similarly suggests that do not have conventional sex roles

Thanks, we have changed the manuscript as follows (lines 347 – 349):

“The latter result indicates that males and females may compete similarly for available mates, suggesting that they do not have conventional sex roles: male-male competition and female choice for mates (Vincent, Ahnesjö, & Berglund, 1994)”

(15) Line 357: Change 'albatross' to 'albatrosses'.

Done, thank you (see line 370)

Reviewer #1:

I am pleased to see that the authors have carefully reviewed this manuscript according to previous suggestions. In my view these changes improved the paper and are sufficient for making the paper a highly interesting contribution within the field of animal mating behaviour.

Thank you

Reviewer #2:

I am satisfied with the changes/improvements done by the authors.

Thank you

1 **HIGHLIGHTS**

2

- 3
- Biparental species mated more quickly than uniparental species
- 4
- Uniparental plover species exhibited sex differences in mating opportunities
- 5
- Courtship behaviour by males differed significantly between plover species
- 6
- Newly established pair bonds were weaker in biparental than in uniparental plovers
- 7
- Mating opportunities and pair bond stability may influence breeding systems

1 **Experimental assessment of mating opportunities in three shorebird species**

2

3 Mating opportunities may differ between closely related species, although the evidence for such
4 variation is scant. Here we compare remating opportunities and courtship behaviour between
5 three shorebird species: the Kentish plover (*Charadrius alexandrinus*), the Kittlitz's plover (*C.*
6 *pecuarius*) and the white-fronted plover (*C. marginatus*), using data and an experimental
7 approach previously developed for the Kentish plover. By experimentally creating unmated
8 males and females, we show that remating opportunities are different between closely related
9 plover species (*Charadrius spp*): remating times were shorter for females than males in a Kentish
10 plover population that exhibits a male-biased adult sex ratio, and where the majority of brood
11 care after hatching is carried out by males. In contrast, remating times were male-biased in the
12 uniparental Kittlitz's plover and unbiased in the biparental white-fronted plover. We also show
13 that male Kentish plovers spend significantly more time on courtship than females, whereas
14 courtship behaviour is not sex biased in the other two plover species. The mate-removal
15 experiments also provided insights into pair bond stability. In the Kittlitz's plover, all 16 newly
16 formed pairs remained together after the release of their former mates from captivity, whereas
17 newly established pairs were replaced by their former mates upon release in 12 out of 12 white
18 fronted plover pairs. Taken together, these results are important in highlighting interspecific
19 variation in mating activities, and suggest that both operational sex ratio (OSR) and pair bond
20 stability may differ between closely related species. These variations in turn, may influence
21 mating systems and parental care.

- 22 *Keywords:* adult sex ratio, mating systems, operational sex ratio, pair bond, parental care,
- 23 remating opportunity.

24 The different evolutionary interests of males and females over reproduction (termed sexual
25 conflict; Parker, 1979) are a pervasive evolutionary force influencing the behaviour, ecology and life
26 histories of many organisms (Arnqvist & Rowe, 2005; Chapman, Arnqvist, Bangham, & Rowe, 2003). A
27 common issue when the interests of males and females are antagonistic concerns offspring care (Trivers,
28 1972; Maynard Smith, 1977; Houston, Székely, & McNamara, 2005; Lessells, 2012). By caring for the
29 offspring, parents often improve the growth and survival of the young (Clutton-Brock, 1991; Klug,
30 Alonzo, & Bonsall, 2012); for example, by gestating, nursing, protecting and feeding the young, the
31 offspring have improved chances of survival (Tyler, Shearman, Franco, O'Brien, Seamark, & Kelly, 1983;
32 Balshine-Earn & Earn 1998; Baeza & Fernández, 2002; Klug, Alonzo, & Bonsall, 2012). However, care
33 is costly in terms of time and energy, and the caring parent can be killed by predators or may lose
34 additional mating opportunities (Veasey, Houston, & Metcalfe, 2001; Li & Jackson, 2003; Klug et al.,
35 2012). Therefore, whilst both biological parents benefit from providing care for the offspring, each parent
36 is expected to withhold his (or her) parental contribution in order to raise further offspring in future
37 (Houston et al., 2005; Lessells, 2012; McGraw, Székely, & Young, 2010).

38 Theory suggests that a key component of conflict resolution between male and female parents is
39 mating opportunity (Székely, Webb, & Cuthill, 2000; McNamara, Székely, Webb, & Houston, 2000;
40 Kokko & Jennions, 2008; Klug et al. 2012). If one sex has more favourable mating opportunities than the
41 other, the former parent is expected to reduce (or completely terminate) care more often than its mate, and
42 seek out a new partner (Balshine-Earn & Earn, 1998; Owens, 2002; Pilastro, Biddau, Marin, & Mingozi,
43 2001). One approach used by researchers to assess mating opportunities is to estimate the ratio of sexually
44 active males to females (operational sex ratio, OSR; Kvarnemo & Ahnesjö, 1996; Forsgren, Amundsen,
45 & Bjelvenmark, 2004). An alternative approach to estimating mating opportunities is to experimentally
46 create unmated individuals, and to quantify their remating behaviour, e.g. time to remate, remating
47 success and reproductive success with the new mate (Lessells, 1983; Székely, Cuthill, & Kis, 1999). This

48 experimental approach is powerful, since it directly assesses the mating potential of unmated individuals
49 at a given time in a population.

50 Here we estimate remating opportunities for two small plover species, the white-fronted plover
51 (*Charadrius marginatus*) and the Kittlitz's plover (*C. pecuarius*), and compare these data with the results
52 of a previous study on the Kentish plover (*C. alexandrinus*, Székely et al., 1999). Small plovers
53 (*Charadrius spp.*) exhibit substantial variation in their breeding systems, since some of these species are
54 monogamous and both parents rear the young, whereas others exhibit polygyny and/ or polyandry
55 whereby a single parent (the male or the female) raises the young to independence (Székely, Thomas, &
56 Cuthill, 2006; Thomas, Székely, & Reynolds, 2007). In addition, plovers typically breed in open areas,
57 and their nests and broods are therefore accessible for experimental manipulations (Székely & Cuthill,
58 2000).

59 A previous experiment established that remating opportunities were female-biased in the Kentish
60 plover (Székely et al., 1999), and this result was consistent with demographic analyses that estimated
61 about 6 times more adult males than females in the population (Kosztolányi, Barta, Küpper, & Székely,
62 2011). Skewed adult sex ratios (ASRs) are common in wild populations (Donald, 2007), and recent works
63 suggest that biased ASRs predict sex roles, mating systems and pair-bonds (Liker, Freckleton, & Székely
64 2013). Here we use an identical experimental protocol in two close relatives of the Kentish plover, the
65 white-fronted plover and Kittlitz's plover, to compare remating opportunities between these three plover
66 species. All three species are insectivorous ground-nesting birds that exhibit similar life-histories and
67 ecology (adult body masses, Kentish plover: 41.8 g; white-fronted plover: 37.1 g; Kittlitz's plover: 35.3 g,
68 Urban, Fry, & Keith, 1986; Hockey, Dean, & Ryan, 2005). The latter two species are common breeding
69 birds in Africa, and their parental care systems differ from the Kentish plover which exhibits male-biased
70 parental care after hatching (Lessells, 1984; Székely & Lessells, 1993; Amat, Fraga, & Arroyo, 1999).
71 White-fronted plovers exhibit biparental brood care, whereas Kittlitz's plovers are reported to exhibit

72 uniparental brood care, carried out by either the male or the female parent (Hockey et al., 2005; Tree,
73 1974; Urban et al., 1986).

74 Based on theoretical models (Klug et al., 2012; Kokko & Jennions, 2008) and available
75 information on patterns of parental care (Hockey et al., 2005; Urban et al., 1986), we derived three
76 predictions. First, we predicted higher remating opportunities in uniparental species (Kittlitz's plover)
77 than in biparental species (white-fronted plover), since in biparental species both parents are engaged with
78 care until the offspring are fully independent (henceforth, **between species comparison**). Second, we
79 predicted no difference in remating opportunities between males and females in biparental white-fronted
80 plover given that both sexes are fully engaged in parental care. Similarly, no difference in remating
81 opportunities between males and females was predicted for the uniparental Kittlitz's plover in which
82 either parent is free to seek a new mate (henceforth, **between sexes comparison**). Third, we predicted
83 intense courtship behaviour by males and females both in biparental white-fronted plover and uniparental
84 Kittlitz's plover where care is provided by either parent (henceforth, **courtship behaviour**). In addition to
85 the experimental assessment of remating opportunities, we also monitored pair bond stability among
86 newly established pairs. We include the Kentish plover in our analyses (using the data from Székely et al.,
87 1999), since the same experimental methodology was used in all three species. Nevertheless, our main
88 conclusions remain consistent when restricting the analyses to the white-fronted and Kittlitz's plovers.

89

90 **METHODS**

91

92 *Study Species and Study Sites*

93

94 White-fronted plovers and the Kittlitz's plovers were investigated in SW Madagascar (for Kentish
95 plover, see details in Székely et al., 1999). Kittlitz's plovers were studied between 6 February 2010 and
96 13 May 2010 in Andavadoaka (22° 02' S, 43° 39' E) where they breed around alkaline lakes.

97 Approximately 300 Kittlitz's plovers breed in Andavadoaka (J.E. Parra, S. Zefania, & T. Székely, unpubl.
98 data). Fieldwork with the white-fronted plover was carried out between 1 April 2011 and 23 June 2011 at
99 Lake Tsimanampetsotsa National Park (24° 3' S, 43°44' E), a large alkaline lake (approx 15 km x 0.5
100 km), surrounded by sandy shores, short grass and saltpans. Approximately 150 white-fronted plovers
101 breed around the lake (J.E. Parra et al., unpubl. data).

102 In the field, we searched for nests on foot, identified incubating parents and watched the parent(s)
103 returning to nests in potential breeding sites. In total, we captured 18 Kittlitz's plover pairs (36
104 individuals) and 14 white-fronted plover pairs (28 individuals) with funnel traps placed on their nests.
105 The traps were continuously monitored until a parent entered the trap and sat on the eggs. Parents were
106 immediately removed from the traps to reduce stress and the risk of injury. Morphological traits (body
107 mass, tarsus length, wing length and bill length) were measured using a spring balance, a sliding calliper
108 and wing ruler (see details in Kentish plover field guide, [www.bath.ac.uk/bio-sci/biodiversity-](http://www.bath.ac.uk/bio-sci/biodiversity-lab/pdfs/KP_Field_Guide_v3.pdf)
109 [lab/pdfs/KP_Field_Guide_v3.pdf](http://www.bath.ac.uk/bio-sci/biodiversity-lab/pdfs/KP_Field_Guide_v3.pdf)). All adults were ringed with an individual combination of colour rings
110 and a numbered SAFRING metal ring from the University of Cape Town, South Africa.

111

112 *Experimental Manipulation*

113

114 We used the methodology developed by Székely et al. (1999) to estimate remating times in the
115 Kentish plover. Briefly, both parents were trapped, ringed, measured and a blood sample was taken for
116 sex determination (see below). One parent was then selected at random (the male or the female) and was
117 released at the capture location immediately. The other parent was taken into captivity (see below). In
118 both Kittlitz's and white-fronted plovers, both the male and female incubate the eggs (Hockey et al.,
119 2005; Urban et al., 1986). Only pairs incubating two eggs (modal clutch size in both species) were
120 manipulated. Egg length and breadth were measured with a sliding calliper, and the number of days the
121 eggs had been incubated for was estimated based on the floatation stage of the egg in a transparent jar

122 with clean water (mean \pm SD no. of days incubated: Kittlitz's plover: 9.0 ± 4.32 days, $N = 36$; white-
123 fronted plover: 11.5 ± 3.16 days, $N = 20$). Eggs were distributed to other non-experimental plover
124 clutches at approximately the same stage of incubation in the local populations. Monitoring the
125 augmented clutches was beyond the scope of the experiment, although casual nest checks suggest that at
126 least 33.3% and 19.4% of augmented nests survived until hatching in the Kittlitz's plover ($N = 36$ nests)
127 and the white-fronted plover ($N = 20$ nests), respectively. Survival in these nests appeared to be higher
128 than for unmanipulated nests (13.4% and 8.9%, based on $N = 101$ Kittlitz's plover nests and $N = 56$
129 white-fronted plover nests, respectively; J.E. Parra et al., unpubl. data).

130 Removed plovers were transported in an air-conditioned vehicle to a purpose-built aviary near the
131 field camp at both study sites. Lightweight bird bags were used to keep the plovers undisturbed and
132 ventilated during the transport. Distance from capture areas to the aviaries varied between 1 and 10 km in
133 both study sites. The aviaries had four units for Kittlitz's plovers and six units for white-fronted plovers.
134 Each unit consisted of a 1 m x 1.5 m x 1.5 m (height x length x width) wood frame fitted with chicken
135 mesh (13 mm x 13 mm). To provide shade for the captive birds, we covered the outside of the aviary with
136 papyrus, *Cyperius sp.*, and fitted 50 cm of cloth at the base of the mesh inside the units. Captive plovers
137 were provided with appropriate food and drink three times every day to maintain their good health using
138 high protein meals: dried invertebrates for wild birds (dried mealworms, dried earthworms, shrimps and
139 dried waterfly; shop.naturesgrub.co.uk/), bird supplement vitamins (Vitacombex V; www.petland.co.uk)
140 and pinhead oatmeal (Prosecto InsectivorousTM; www.haiths.com). Captive plovers were also supplied
141 with fresh insects twice a day using pit fall traps set-up in the salt-marsh. In addition, two water recipients
142 were set for drinking and bathing in each unit. Captive plovers were released after their former mate
143 found a new mate or their former mate was not seen in the study sites for at least 12 days. Time in
144 captivity was comparable between white-fronted plovers (mean \pm SD no. of days in captivity: 8.0 ± 1.71
145 days, $N = 14$) and Kittlitz's plovers (7.12 ± 2.57 days, $N = 18$). Captive plovers were measured before
146 release. Although captive plovers appeared to lose a small amount of body mass during their time in

147 captivity (2.77 ± 0.51 g in Kittlitz's plover, and 0.73 ± 0.22 g in white-fronted plover), they were in good
148 condition as indicated by the fact that many remated shortly after release from captivity (see Results).

149

150 *Behavioural Observations*

151

152 The released plovers were searched for every day in the field using a car and mobile hide. When a
153 focal plover was found, we recorded its behaviour for 30 min at 30 seconds intervals. Attempts were
154 made to record the behaviour of focal plovers on at least two occasions before they found a new mate. 30
155 min is sufficient to establish whether a plover is mated or not (Székely et al., 1999). We used behavioural
156 categories of courting and self-maintenance behaviours that were previously developed for the Kentish
157 plover (Székely et al., 1999). Courtship behaviours included: (1) Courting: male plovers perform upright
158 posture and high-stepping movements and female plovers perform a lower head position. (2) Copulations:
159 courting pairs frequently copulated. (3) Scraping: male and female plovers dig several scrapes in a
160 territory. (4) Fighting: focal individuals chase away intruders with buffed-out plumage and buzzing calls.
161 Self-maintenance behaviours included: (1) Feeding: individuals pick up food items followed by a short
162 run; (2) Preening: an individual groomed its own feathers. Mated individuals were identified based on
163 courtship behaviours including courting, scrape ceremony and copulation (Urban et al., 1986; Hockey et
164 al., 2005, see video of Kittlitz's plover courting behaviour in the Supplementary Material, Video S1).
165 New pairs were checked for clutches every day, and eggs were measured as described above. Two
166 observers recorded all behavioural observations (M. Beltrán and J.E. Parra).

167

168 *Molecular Sexing*

169

170 Both plover species have sexually monomorphic plumage (Hockey et al., 2005; Urban et al.,
171 1986), therefore we used molecular sex-typing to determine the sex of individuals (dos Remedios, Lee,

172 Székely, Dawson, & Küpper, 2010). A small blood sample was taken from each adult's brachial wing
173 vein, by puncturing, collecting drops of blood (~25 ul) in capillary tubes, and storing this in Eppendorf
174 tubes of Queen's Lysis Buffer. DNA was extracted from blood samples using the Ammonium Acetate
175 extraction method (Miller, Dykes, & Polesky, 1988; Richardson, Jury, Blaakmeer, Komdeur, & Burke,
176 2001). For molecular sex-typing, Z- and W-chromosome specific genes were amplified via polymerase
177 chain reaction (PCR) using the Z-002B/Z-002D primers (Dawson, 2007). For additional certainty in sex
178 assignment, the W-chromosome specific Calex-31 primers, developed in the genus *Charadrius* were
179 utilized (Küpper, Horsburgh, Dawson, Ffrench-Constant, Székely, & Burke, 2006). PCR amplification
180 was conducted on a DNA Engine Tetrad 2 Peltier Thermal Cycler under the following conditions: 95°C
181 for 15 min, followed by 35 cycles of 94°C for 30 s, 56°C for 90 s, 72°C for 60 s with a final extension of
182 60°C for 30 min. Samples were visualized on an ABI 3730 automated sequencer. IR Dye-labelled tailed
183 primers separated the products of Z-002B/Z-002D primers into either one (ZZ) or two bands (ZW),
184 indicating male or female respectively. The W-specific Calex-31 product appeared as one band indicating
185 female only. Images were scored using GeneMapper software version 4.1 (Applied Biosystems). To
186 maximize reliability, all samples were sexed using two markers. Additionally, for 8% of samples (11
187 Kittlitz's plover and 10 white-fronted plover individuals) molecular sexing was repeated; in all cases,
188 repetitions concurred with the original results.

189

190 *Data Processing and Statistical Analyses*

191

192 Date of mating was defined as the mean date between the date when a plover was last seen single
193 and the first date it was seen with a new mate. Remating time was the difference between date of release
194 (either on the day of manipulation or from captivity) and date of mating. The response variable, remating
195 time, was analyzed using generalized linear models (GLM) with Tweedie (1.5) error structure and a log
196 link function (Smyth & Verbyla, 1999). The models investigated the effects of two main variables:

197 species (Kentish, white-fronted and Kittlitz's plovers) and sex; and three additional fixed variables: type
198 of manipulation (released in the field or released from captivity), release date, and number of days in
199 captivity (see Table 1). Dates were expressed as Julian dates, i.e. number of days since 1 January. Results
200 of backward elimination based on Akaike's information criterion for small sample sizes (AICc) are
201 presented for variable selection of the GLM models where lowest AICc score is the best supported model
202 (Symonds & Moussalli, 2010; Table 1). Pairwise multiple comparisons were performed to compare mean
203 differences of mating time between males and females of the three species using Fisher's least significant
204 difference tests (LSD test).

205 Remating time was also analyzed using survival analyses and these estimates are referred to as
206 expected remating times (see rationale in Székely et al., 1999). In these analyses, the terminal event
207 (outcome) was the occurrence of mating, defined as the first observation when a plover was seen with a
208 mate. Several individuals did not find a new mate when we saw them for the last time, and these were
209 treated as censored observations. First, we used a Gehan-Wilcoxon test to compare expected remating
210 times curves (survival curves) for three species by sex. Survival curves were generated by the Kaplan-
211 Meier method. Second, for each plover species a separate Cox regression model was constructed to
212 investigate the probability of remaining single from the day of release (season), sex and their interaction
213 as covariates (Table 2).

214 Courting behaviour was estimated as percentage obtained from each 30 min sample. For
215 individuals with several behavioural observations, we calculated the mean percentage of courting.
216 Courting behaviour was analyzed using GLMs with Tweedie (1.5) error structure and a logarithmic link
217 function per individual plover. The model included two main factors: species of plover and sex; and three
218 additional fixed variables: type of manipulation (released in the field or from captivity), released date, and
219 number of days in captivity. Model selection and statistical parameters estimated for each independent
220 variable in the models are provided in the Appendix, Table A1. In addition, for each sex a separate GLM
221 model was constructed to investigate the effect of three species of plovers on courting behaviour (Table

222 3). Pairwise multiple comparisons were performed to compare mean differences of courtship behaviour
223 between males and females in the three species of plovers using LSD tests.

224 Finally, pair bond stability was analysed comparing the frequencies of mate replacement between
225 white-fronted plover and Kittlitz's plover with Fisher's exact test. Data were analyzed by using SPSS
226 statistics for Windows version 19 and figures were made in R (R Development Core Team, 2008) using
227 the package ggplot2 (Wickham, 2009).

228

229 *Ethical Note*

230

231 The experiments in Madagascar were approved by the Ministry of Environment, Forests and
232 Tourism of the Republic of Madagascar (Research permit No: 053/11/MEF/SG/DGF/DCB.SAP/SCB of
233 11 March 2011 and 132/10/MEF/SG/DGF/DCB.SAP/SSE of 06 May 2010) and Madagascar National
234 Parks (No: 398-10/MEF/SG/DGF/DVRN/SGFF of 18 May 2011). Blood sampling was also covered by
235 these research permits. The blood transport permit was approved by Service de la Gestion de la Faune et
236 de la Flore, Direction de la Valorisation des Ressources Naturelles, Ministère de l'Environnement et des
237 Forêts Madagascar (authorization number 080N-EA06/MG11). The Kentish plover experiment was
238 approved by the Turkish Ministry of Environment (see Székely et al., 1999). The Kittlitz's and the white-
239 fronted plovers are common breeding birds in much of Africa and Madagascar and they are not
240 considered threatened by the IUCN (BirdLife International, 2012). Captive plovers were monitored daily
241 and kept under standard conditions (see Experimental Manipulation) to reduce their stress levels. In
242 addition, translocated eggs coped with the natural breeding conditions of local clutches in the two plover
243 populations (see above). The experiment was designed to reduce adverse effects on plover welfare and
244 their local populations.

245

246 **RESULTS**

247

248 *Between Species Comparison*

249

250 Remating opportunities differed significantly between the three plover species (Fig. 1): white-
251 fronted plovers mated significantly more quickly (median = 2.0 days, range 0.5 – 4.5 days, $N = 12$) than
252 both Kittlitz's plovers (median = 5.1 days, range 1.0 – 11.5 days, $N = 16$) and Kentish plovers (median =
253 6.3 days, range 0.5 – 47.5 days, $N = 34$; Table 1).

254 These results remained consistent using survival analyses that also included the individuals that
255 were not successful in finding a new mate (Fig. 2, Table 2; see Methods). The proportion of plovers
256 remaining single was significantly lower for the white-fronted plover (median = 4 days, $N = 14$) than both
257 the Kittlitz's plover (median = 14.6 days, $N = 33$) and Kentish plover (median = 13.4 days, $N = 59$; testing
258 the three species, Wilcoxon–Gehan test: $\chi^2_2 = 16.316$, $P < 0.001$).

259

260 *Between Sexes Comparison*

261

262 A significant species by sex interaction suggested a sex-biased difference in remating
263 opportunities (GLM: $\chi^2_2 = 47.62$, $P < 0.001$, Table 1). Female Kittlitz's plovers took significantly longer
264 to mate (median = 6.5 days, range 3.5 – 11.5 days, $N = 6$) than males (median = 3.3, range 1.0 – 7.5 days,
265 $N = 10$; LSD test: pairwise mean difference = -0.66, $df = 1$, $P = 0.047$) whereas the opposite was found in
266 the Kentish plover (Székely et al. 1999). However, male and female remating times were not significantly
267 different in white-fronted plovers (male: median: 2.0 days, range 0.5 – 3.5 days, $N = 6$; female: median:
268 2.0 days, 1.0 – 4.5 days, $N = 6$, LSD test: pairwise mean difference = -0.11, $df = 1$, $P = 0.823$).

269 These results remained consistent using survival analyses (Table 2): the proportion of female
270 Kittlitz's plovers remaining single was higher than that of males (male median: 11.0 days, $N = 17$, female

271 median: 21.0 days, $N = 16$, Fig. 2), whereas the proportion of single males and females were not
272 significantly different in the white-fronted plover (male median: 3.0 days, $N = 7$, female median: 4.0 days,
273 $N = 7$, Fig. 2). Remating time increased over the season only for female Kentish plovers (Cox regression:
274 $\chi^2_1 = 7.66$, $P = 0.014$), suggesting an influence of time of breeding season on mating opportunities in the
275 Kentish plover, although this was not the case in the other two species (Table 2).

276

277 *Courtship Behaviour*

278

279 Courtship behaviour had a significant species by sex interaction (GLM: $\chi^2_2 = 6.329$, $P = 0.042$,
280 Supplementary Material: Table S2, Fig. 3). Courtship behaviour by males differed significantly between
281 species (GLM: $\chi^2_2 = 10.689$, $P = 0.005$, Table 3), male Kentish plovers spent significantly more time on
282 courtship than males of the other plover species; whereas courtship behaviour by females did not differ
283 between species (GLM: $\chi^2_2 = 1.437$, $P = 0.487$, Table 3). In contrast to the Kentish plover, which
284 exhibited male-biased courtship behaviour (LSD test: pairwise mean difference = -3.29, $df = 1$, $P =$
285 **0.005**), males and females of the other two species spent comparable times on courtship (LSD test white-
286 fronted plover: pairwise mean difference = 1.06, $df = 1$, $P = 0.252$; LSD test Kittlitz's plover: pairwise
287 mean difference = -0.36, $df = 1$, $P = 0.679$, Fig. 3).

288

289 *Pair Bonds*

290

291 The new pair bonds in experimentally-induced white-fronted plovers were significantly weaker
292 than in Kittlitz's plover: in 12 white-fronted plovers that remated after their former partner was removed
293 (6 males, 6 females), all experimentally-induced pair bonds were replaced by the original mates after they
294 were released from captivity. In contrast, in 16 Kittlitz's plovers that remated after their former partner

295 was removed (10 male and 6 female), none were replaced by their former mates once their former mates
296 were released from captivity (Fisher's exact test: mate replacement in white-fronted plover =12, $N = 12$;
297 mate replacement in Kittlitz's plover = 0, $N = 16$; $P < 0.001$)

298

299 **DISCUSSION**

300

301 These experiments provided four key results. First, they show that mating opportunities are
302 significantly different between closely related species. This result is striking because two of these plover
303 species (white-fronted and Kittlitz's) breed in the same habitat in Madagascar, and therefore, ecological
304 factors are unlikely to explain the differences in remating opportunities. The rapid remating of white-
305 fronted plovers – a pattern we did not expect – suggests that there is a large pool of floating individuals
306 that can rapidly move in to pair up with unmated individuals. Consistent with the latter argument, both
307 male and female white-fronted plovers stayed in the same territory, and new individuals moved in to
308 replace the removed mates. Mating opportunities seem to be an important factor in the evolution of
309 breeding systems across a range of species (Balshine-Earn & Earn, 1998; Magrath & Komdeur, 2003).
310 For example, as in the white-fronted plover, male dunlin (*Calidris alpina*) were rapidly replaced by other
311 males after experimental removal from their breeding territories (Holmes, 1970; Pitelka, Holmes, &
312 Maclean, 1974). In the European starling (*Sturnus vulgaris*), after mating opportunities were increased by
313 the provision of additional nest-boxes, males increased their mating effort to attract additional mates and
314 also reduced parental care effort (Smith, 1995), and in St. Peter's fish (*Sarotherodon galilaeus*), males
315 and females were more likely to desert the offspring when remating opportunities were increased
316 experimentally (Balshine-Earn & Earn, 1998).

317 Second, we found sex-bias in remating opportunities: the male-biased remating opportunities in
318 Kittlitz's plover were the opposite of those found in the Kentish plover (Székely et al., 1999), whereas in
319 white-fronted plovers remating opportunities did not differ between males and females. As far as we are

320 aware, our study is the first to experimentally demonstrate differences in sex-biased remating
321 opportunities between closely related species in wild populations. Sex-biased mating opportunities may
322 emerge in two ways. One explanation is that the ratio of sexually active males to females (operational sex
323 ratio, OSR) may not be at parity. OSR may be biased due to differences in the reproductive schedules of
324 males and females (“time in”: time spent in the mating pool, sexually active; “time out”: time spent out of
325 the mating pool, not sexually active), or biased adult sex ratios (ASR; Donald, 2007). Evidence suggests
326 that OSR can vary due to mating and parental care activities (Forsgren et al., 2004, Symons, Svensson, &
327 Wong, 2011; LaBarbera, Lovette, & Llambías, 2011; Canal, Jovani, & Potti, 2012). In addition, recent
328 studies found substantial difference in ASR between closely related shorebird species (Liker et al., 2013).
329 Further works are needed to separate whether biased remating opportunities emerge via different
330 reproductive scheduling or biased ASR in plovers. For one of these species, the Kentish plover, a
331 demographic study confirmed male-biased ASR (Kosztolányi et al., 2011), although ASR has not been
332 estimated for the white-fronted and the Kittlitz’s plover. Alternatively, sex-biased mating opportunities
333 may arise due to differences in the willingness of males and females to remate. For instance, the post-
334 breeding refractory periods, the recovery phase spent preparing for another breeding attempt, may differ
335 between males and females (Balshine-Earn & Earn, 1998; Cantoni & Brown, 1997): females typically
336 need more time to recover than do males. However, the latter explanation is unlikely, since the adult
337 plovers used in our experiments had breeding efforts interrupted and sought new mates shortly after
338 removal of their mate (or on release from captivity). Furthermore, several female Kentish plovers remated
339 within less than a day – a pattern that is inconsistent with the explanation that females need more time to
340 recover than do males.

341 Third, male courtship behaviour was different between the three species, since male Kentish
342 plovers spent more time on courtship than male white-fronted and Kittlitz’s plovers. This pattern is
343 consistent with the explanation that ASR is male-biased in the Kentish plover. The significance of this
344 result is that courtship behaviour is variable between closely related species and suggests that ASR, and in

345 turn the OSR, is probably related to the intensity of mating competition in males. Consequently,
346 comparable intensity of courtship behaviour was observed between sexes in the Kittlitz's and white-
347 fronted plovers. The latter result indicates that males and females may compete similarly for available
348 mates, suggesting that they do not have conventional sex roles: male-male competition and female choice
349 for mates (Vincent, Ahnesjö, & Berglund, 1994). Variation in resources for breeding has also been
350 suggested to influence OSR, and in turn, the intensity of mating competition (Forsgren, Kvarnemo, &
351 Lindstrom, 1996). Availability of breeding territories, for example, may affect the OSR, since the sex that
352 holds the territories will be limited by scarcity of nest sites. In a sand goby population (*Pomatoschistus*
353 *minutes*), for instance, nest-site abundance can influence the intensity of male mating competition
354 (Forsgren et al., 1996). Hence, the dynamic of OSR, and in turn mating competition, is probably
355 modulated by both ASR and resource availability (Kvarnemo & Ahnesjö, 1996, Forsgren et al., 2004).

356 Finally, the new pair bonds were significantly weaker in white-fronted plovers than in Kittlitz's
357 plover suggesting that the former species exhibits long-term pair bonds whereas the latter has short-term
358 pair bonds. Mate fidelity may emerge in two ways. On the one hand, former mates may actively seek out
359 each other *per se*, and prefer to mate with each other. On the other hand, mate fidelity may emerge via
360 site fidelity: white-fronted plovers are highly territorial (Lloyd, 2008), and therefore upon release from
361 captivity, individuals return to their former territories and chase out their former partner's new mates.
362 Established pairs may prefer to reunite because of the fitness benefits in terms of synchronisation of
363 behavioural and physiological characteristics such as defence of breeding territories, courtship
364 behaviours, laying date, incubation, chick-raising between others which have been shown to improve with
365 time and experience of the pair (Bried, Pontier, & Jouventin, 2003; Rowley, 1983). An experiment carried
366 out in bearded reedling *Panurus biarmicus* found that long-term pair bond formation improved
367 coordination of breeding activities and reproductive success (Griggio & Hoi, 2011). In another example,
368 newly formed pairs (either due to divorce or loss of a mate) had lower reproductive success than
369 established pairs in black turnstone *Arenaria melanocephala* (Handel & Gill, 2000). Improved breeding

370 with increased experience is also well known in geese, albatrosses and other long-term pair bonding
371 animals (Angelier, Weimerskirch, Dano, & Chastel, 2006; Black, 2001). Overall, the consequences of
372 pair bond and site fidelity on mating opportunities could be significant since the ability of an individual to
373 mate may be limited by their access to mates and breeding sites.

374 In conclusion, using an experimental approach we found significant differences in remating
375 opportunities between closely related plover species. As mating opportunity is linked to OSR and ASR,
376 our work suggests that substantial variation in OSR (and possibly ASR) is exhibited among closely
377 related species. Such variation may influence the direction and intensity of competition in males and
378 females for mates and breeding territories. These differences in OSR, in turn, may facilitate different
379 intensities of sexual selection and induce different mating systems and patterns of parental care.

380

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529 **Figure Legends**

530

531 **Figure 1.** Remating times in three plover species. The lower and upper borders of the box are lower and
532 upper quartiles, respectively, the horizontal bar is the median and whiskers represent the lowest and
533 highest observations.

534

535 **Figure 2.** Proportion of males and females remaining single in three plover species: Kentish plover (top),
536 white-fronted plover (middle) and Kittlitz's plover (bottom). Dotted lines show the expected mating time
537 of males and females after release. Number of individuals: 32 male and 27 female Kentish plovers; 7 male
538 and 7 female white-fronted plovers; and 17 male and 16 female Kittlitz's plovers.

539

540 **Figure 3.** Courtship behaviour in three plover species. The lower and upper borders of the box are lower
541 and upper quartiles, respectively, the horizontal bar is the median and whiskers represent the lowest and
542 highest observations. Circles denote outliers that are between the first and third interquartile from the
543 nearer edge of the box.

544 **Table 1.**

545 Remating times (response variable) of males and females in three species of plover.

Independent variable	Full model (AICc = 346.004)			Best model (AICc = 341.088)		
	Wald χ^2	Df	P	Wald χ^2	Df	P
(Intercept)	38.596	1	<0.001	49.365	1	<0.001
Between species comparison						
Species	11.248	2	0.004	11.595	2	0.003
Between sexes comparison						
Sex	4.072	1	0.044	3.974	1	0.046
Species * sex	39.65	2	<0.001	47.620	2	<0.001
Manipulation	0.290	1	0.59	-	-	-
Release date	4.818	1	0.028	5.007	1	0.025
Captive days	0.646	1	0.422	-	-	-

546 GLMs were used to analyze mating time using Tweedie (1.5) error structure and a log link function.

547 Model selection was carried out using Akaike information criterion for small sample sizes (AICc).

548 Manipulation refers to whether a plover was kept in captivity or not. Release date refers to the date when
549 a plover was released to find a new mate (Julian dates). Captive days are the number of days that a plover
550 was kept in captivity.

551

552 **Table 2.**

553 Remating time in plovers based on Cox proportional hazard models, to examine the relationship of the
 554 survival distribution which includes censored observations.

Species	Variable	B	Wald χ^2	df	p
Kentish plover	Sex	1.541	12.07	1	0.001
	Release date	-0.024	6.073	1	0.014
White-fronted plover	Sex	-0.18	0.083	1	0.77
	Release date	0.002	0.004	1	0.95
Kittlitz's plover	Sex	-1.342	4.864	1	0.027
	Release date	-0.01	0.088	1	0.767

555 For each species a separate model was constructed. Number of individual Kentish plovers, mated = 34,
 556 censored = 19; white-fronted plover, 12, 2; Kittlitz's plover, 16, 17, respectively.

557

558 **Table 3.**

559 Courtship behaviour (response variable: % of time courting) in three plover species.

Sex	Variables	Wald χ^2	<i>Df</i>	<i>P</i>
Male	(Intercept)	13.176	1	< 0.001
	Species	10.689	2	0.005
Female	(Intercept)	0.155	1	0.694
	Species	1.437	2	0.487

560 GLMs were used to analyse percentage of time courting using Tweedie (1.5) error structure and a log link

561 function. Model selection was carried out using Akaike information criterion for small sample sizes

562 (AICc).

563 **APPENDIX**

564 **Table A1.**

565 Courtship behaviour in plovers (response variable: proportion of time courting).

Independent variable	Full model (AICc = 269.035)			Best model (AICc = 261.316)		
	Wald χ^2	d.f.	p	Wald χ^2	d.f.	P
(Intercept)	2.215	1	0.137	2.914	1	0.088
Species	0.621	2	0.733	0.791	2	0.673
Sex	5.713	1	0.017	5.381	1	0.020
Species * sex	6.084	2	0.048	6.329	2	0.042
Manipulation	0.057	1	0.811	-	-	-
Release date	0.056	1	0.813	-	-	-
Captive days	0.477	1	0.490	-	-	-

566 GLMs were used to analyse courtship behaviour using Tweedie (1.5) error structure and a log link
 567 function. Model selection was carried out using Akaike information criterion for small sample sizes
 568 (AICc).

1 **Experimental assessment of mating opportunities in three shorebird species**

2

3 Mating opportunities may differ between closely related species, although the evidence for such
4 variation is scant. Here we compare remating opportunities and courtship behaviour between
5 three shorebird species: the Kentish plover (*Charadrius alexandrinus*), the Kittlitz's plover (*C.*
6 *pecuarius*) and the white-fronted plover (*C. marginatus*), using data and an experimental
7 approach previously developed for the Kentish plover. By experimentally creating unmated
8 males and females, we show that remating opportunities are different between closely related
9 plover species (*Charadrius spp*): remating times were shorter for females than males in a Kentish
10 plover population that exhibits a male-biased adult sex ratio, and where the majority of brood
11 care after hatching is carried out by males. In contrast, remating times were male-biased in the
12 uniparental Kittlitz's plover and unbiased in the biparental white-fronted plover. We also show
13 that male Kentish plovers spend significantly more time on courtship than females, whereas
14 courtship behaviour is not sex biased in the other two plover species. The mate-removal
15 experiments also provided insights into pair bond stability. In the Kittlitz's plover, all 16 newly
16 formed pairs remained together after the release of their former mates from captivity, whereas
17 newly established pairs were replaced by their former mates upon release in 12 out of 12 white
18 fronted plover pairs. Taken together, these results are important in highlighting interspecific
19 variation in mating activities, and suggest that both operational sex ratio (OSR) and pair bond
20 stability may differ between closely related species. These variations in turn, may influence
21 mating systems and parental care.

- 22 *Keywords:* adult sex ratio, mating systems, operational sex ratio, pair bond, parental care,
23 remating opportunity.

24 The different evolutionary interests of males and females over reproduction (termed sexual
25 conflict; Parker, 1979) are a pervasive evolutionary force influencing the behaviour, ecology and life
26 histories of many organisms (Arnqvist & Rowe, 2005; Chapman, Arnqvist, Bangham, & Rowe, 2003). A
27 common issue when the interests of males and females are antagonistic concerns offspring care (Trivers,
28 1972; Maynard Smith, 1977; Houston, Székely, & McNamara, 2005; Lessells, 2012). By caring for the
29 offspring, parents often improve the growth and survival of the young (Clutton-Brock, 1991; Klug,
30 Alonzo, & Bonsall, 2012); for example, by gestating, nursing, protecting and feeding the young, the
31 offspring have improved chances of survival (Tyler, Shearman, Franco, O'Brien, Seamark, & Kelly, 1983;
32 Balshine-Earn & Earn 1998; Baeza & Fernández, 2002; Klug, Alonzo, & Bonsall, 2012). However, care
33 is costly in terms of time and energy, and the caring parent can be killed by predators or may lose
34 additional mating opportunities (Veasey, Houston, & Metcalfe, 2001; Li & Jackson, 2003; Klug et al.,
35 2012). Therefore, whilst both biological parents benefit from providing care for the offspring, each parent
36 is expected to withhold his (or her) parental contribution in order to raise further offspring in future
37 (Houston et al., 2005; Lessells, 2012; McGraw, Székely, & Young, 2010).

38 Theory suggests that a key component of conflict resolution between male and female parents is
39 mating opportunity (Székely, Webb, & Cuthill, 2000; McNamara, Székely, Webb, & Houston, 2000;
40 Kokko & Jennions, 2008; Klug et al. 2012). If one sex has more favourable mating opportunities than the
41 other, the former parent is expected to reduce (or completely terminate) care more often than its mate, and
42 seek out a new partner (Balshine-Earn & Earn, 1998; Owens, 2002; Pilastro, Biddau, Marin, & Mingozi,
43 2001). One approach used by researchers to assess mating opportunities is to estimate the ratio of sexually
44 active males to females (operational sex ratio, OSR; Kvarnemo & Ahnesjö, 1996; Forsgren, Amundsen,
45 & Bjelvenmark, 2004). An alternative approach to estimating mating opportunities is to experimentally
46 create unmated individuals, and to quantify their remating behaviour, e.g. time to remate, remating
47 success and reproductive success with the new mate (Lessells, 1983; Székely, Cuthill, & Kis, 1999). This

48 experimental approach is powerful, since it directly assesses the mating potential of unmated individuals
49 at a given time in a population.

50 Here we estimate remating opportunities for two small plover species, the white-fronted plover
51 (*Charadrius marginatus*) and the Kittlitz's plover (*C. pecuarius*), and compare these data with the results
52 of a previous study on the Kentish plover (*C. alexandrinus*, Székely et al., 1999). Small plovers
53 (*Charadrius spp.*) exhibit substantial variation in their breeding systems, since some of these species are
54 monogamous and both parents rear the young, whereas others exhibit polygyny and/ or polyandry
55 whereby a single parent (the male or the female) raises the young to independence (Székely, Thomas, &
56 Cuthill, 2006; Thomas, Székely, & Reynolds, 2007). In addition, plovers typically breed in open areas,
57 and their nests and broods are therefore accessible for experimental manipulations (Székely & Cuthill,
58 2000).

59 A previous experiment established that remating opportunities were female-biased in the Kentish
60 plover (Székely et al., 1999), and this result was consistent with demographic analyses that estimated
61 about 6 times more adult males than females in the population (Kosztolányi, Barta, Küpper, & Székely,
62 2011). Skewed adult sex ratios (ASRs) are common in wild populations (Donald, 2007), and recent works
63 suggest that biased ASRs predict sex roles, mating systems and pair-bonds (Liker, Freckleton, & Székely
64 2013). Here we use an identical experimental protocol in two close relatives of the Kentish plover, the
65 white-fronted plover and Kittlitz's plover, to compare remating opportunities between these three plover
66 species. All three species are insectivorous ground-nesting birds that exhibit similar life-histories and
67 ecology (adult body masses, Kentish plover: 41.8 g; white-fronted plover: 37.1 g; Kittlitz's plover: 35.3 g,
68 Urban, Fry, & Keith, 1986; Hockey, Dean, & Ryan, 2005). The latter two species are common breeding
69 birds in Africa, and their parental care systems differ from the Kentish plover which exhibits male-biased
70 parental care after hatching (Lessells, 1984; Székely & Lessells, 1993; Amat, Fraga, & Arroyo, 1999).
71 White-fronted plovers exhibit biparental brood care, whereas Kittlitz's plovers are reported to exhibit

72 uniparental brood care, carried out by either the male or the female parent (Hockey et al., 2005; Tree,
73 1974; Urban et al., 1986).

74 Based on theoretical models (Klug et al., 2012; Kokko & Jennions, 2008) and available
75 information on patterns of parental care (Hockey et al., 2005; Urban et al., 1986), we derived three
76 predictions. First, we predicted higher remating opportunities in uniparental species (Kittlitz's plover)
77 than in biparental species (white-fronted plover), since in biparental species both parents are engaged with
78 care until the offspring are fully independent (henceforth, between species comparison). Second, we
79 predicted no difference in remating opportunities between males and females in biparental white-fronted
80 plover given that both sexes are fully engaged in parental care. Similarly, no difference in remating
81 opportunities between males and females was predicted for the uniparental Kittlitz's plover in which
82 either parent is free to seek a new mate (henceforth, between sexes comparison). Third, we predicted
83 intense courtship behaviour by males and females both in biparental white-fronted plover and uniparental
84 Kittlitz's plover where care is provided by either parent (henceforth, courtship behaviour). In addition to
85 the experimental assessment of remating opportunities, we also monitored pair bond stability among
86 newly established pairs. We include the Kentish plover in our analyses (using the data from Székely et al.,
87 1999), since the same experimental methodology was used in all three species. Nevertheless, our main
88 conclusions remain consistent when restricting the analyses to the white-fronted and Kittlitz's plovers.

89

90 **METHODS**

91

92 *Study Species and Study Sites*

93

94 White-fronted plovers and the Kittlitz's plovers were investigated in SW Madagascar (for Kentish
95 plover, see details in Székely et al., 1999). Kittlitz's plovers were studied between 6 February 2010 and
96 13 May 2010 in Andavadoaka (22° 02' S, 43° 39' E) where they breed around alkaline lakes.

97 Approximately 300 Kittlitz's plovers breed in Andavadoaka (J.E. Parra, S. Zefania, & T. Székely, unpubl.
98 data). Fieldwork with the white-fronted plover was carried out between 1 April 2011 and 23 June 2011 at
99 Lake Tsimanampetsotsa National Park (24° 3' S, 43°44' E), a large alkaline lake (approx 15 km x 0.5
100 km), surrounded by sandy shores, short grass and saltpans. Approximately 150 white-fronted plovers
101 breed around the lake (J.E. Parra et al., unpubl. data).

102 In the field, we searched for nests on foot, identified incubating parents and watched the parent(s)
103 returning to nests in potential breeding sites. In total, we captured 18 Kittlitz's plover pairs (36
104 individuals) and 14 white-fronted plover pairs (28 individuals) with funnel traps placed on their nests.
105 The traps were continuously monitored until a parent entered the trap and sat on the eggs. Parents were
106 immediately removed from the traps to reduce stress and the risk of injury. Morphological traits (body
107 mass, tarsus length, wing length and bill length) were measured using a spring balance, a sliding calliper
108 and wing ruler (see details in Kentish plover field guide, [www.bath.ac.uk/bio-sci/biodiversity-](http://www.bath.ac.uk/bio-sci/biodiversity-lab/pdfs/KP_Field_Guide_v3.pdf)
109 [lab/pdfs/KP_Field_Guide_v3.pdf](http://www.bath.ac.uk/bio-sci/biodiversity-lab/pdfs/KP_Field_Guide_v3.pdf)). All adults were ringed with an individual combination of colour rings
110 and a numbered SAFRING metal ring from the University of Cape Town, South Africa.

111

112 *Experimental Manipulation*

113

114 We used the methodology developed by Székely et al. (1999) to estimate remating times in the
115 Kentish plover. Briefly, both parents were trapped, ringed, measured and a blood sample was taken for
116 sex determination (see below). One parent was then selected at random (the male or the female) and was
117 released at the capture location immediately. The other parent was taken into captivity (see below). In
118 both Kittlitz's and white-fronted plovers, both the male and female incubate the eggs (Hockey et al.,
119 2005; Urban et al., 1986). Only pairs incubating two eggs (modal clutch size in both species) were
120 manipulated. Egg length and breadth were measured with a sliding calliper, and the number of days the
121 eggs had been incubated for was estimated based on the floatation stage of the egg in a transparent jar

122 with clean water (mean \pm SD no. of days incubated: Kittlitz's plover: 9.0 ± 4.32 days, $N = 36$; white-
123 fronted plover: 11.5 ± 3.16 days, $N = 20$). Eggs were distributed to other non-experimental plover
124 clutches at approximately the same stage of incubation in the local populations. Monitoring the
125 augmented clutches was beyond the scope of the experiment, although casual nest checks suggest that at
126 least 33.3% and 19.4% of augmented nests survived until hatching in the Kittlitz's plover ($N = 36$ nests)
127 and the white-fronted plover ($N = 20$ nests), respectively. Survival in these nests appeared to be higher
128 than for unmanipulated nests (13.4% and 8.9%, based on $N = 101$ Kittlitz's plover nests and $N = 56$
129 white-fronted plover nests, respectively; J.E. Parra et al., unpubl. data).

130 Removed plovers were transported in an air-conditioned vehicle to a purpose-built aviary near the
131 field camp at both study sites. Lightweight bird bags were used to keep the plovers undisturbed and
132 ventilated during the transport. Distance from capture areas to the aviaries varied between 1 and 10 km in
133 both study sites. The aviaries had four units for Kittlitz's plovers and six units for white-fronted plovers.
134 Each unit consisted of a 1 m x 1.5 m x 1.5 m (height x length x width) wood frame fitted with chicken
135 mesh (13 mm x 13 mm). To provide shade for the captive birds, we covered the outside of the aviary with
136 papyrus, *Cyperius sp.*, and fitted 50 cm of cloth at the base of the mesh inside the units. Captive plovers
137 were provided with appropriate food and drink three times every day to maintain their good health using
138 high protein meals: dried invertebrates for wild birds (dried mealworms, dried earthworms, shrimps and
139 dried waterfly; shop.naturesgrub.co.uk/), bird supplement vitamins (Vitacombex V; www.petland.co.uk)
140 and pinhead oatmeal (Prosecto InsectivorousTM; www.haiths.com). Captive plovers were also supplied
141 with fresh insects twice a day using pit fall traps set-up in the salt-marsh. In addition, two water recipients
142 were set for drinking and bathing in each unit. Captive plovers were released after their former mate
143 found a new mate or their former mate was not seen in the study sites for at least 12 days. Time in
144 captivity was comparable between white-fronted plovers (mean \pm SD no. of days in captivity: 8.0 ± 1.71
145 days, $N = 14$) and Kittlitz's plovers (7.12 ± 2.57 days, $N = 18$). Captive plovers were measured before
146 release. Although captive plovers appeared to lose a small amount of body mass during their time in

147 captivity (2.77 ± 0.51 g in Kittlitz's plover, and 0.73 ± 0.22 g in white-fronted plover), they were in good
148 condition as indicated by the fact that many remated shortly after release from captivity (see Results).

149

150 *Behavioural Observations*

151

152 The released plovers were searched for every day in the field using a car and mobile hide. When a
153 focal plover was found, we recorded its behaviour for 30 min at 30 seconds intervals. Attempts were
154 made to record the behaviour of focal plovers on at least two occasions before they found a new mate. 30
155 min is sufficient to establish whether a plover is mated or not (Székely et al., 1999). We used behavioural
156 categories of courting and self-maintenance behaviours that were previously developed for the Kentish
157 plover (Székely et al., 1999). Courtship behaviours included: (1) Courting: male plovers perform upright
158 posture and high-stepping movements and female plovers perform a lower head position. (2) Copulations:
159 courting pairs frequently copulated. (3) Scraping: male and female plovers dig several scrapes in a
160 territory. (4) Fighting: focal individuals chase away intruders with buffed-out plumage and buzzing calls.
161 Self-maintenance behaviours included: (1) Feeding: individuals pick up food items followed by a short
162 run, (2) Preening: an individual groomed its own feathers. Mated individuals were identified based on
163 courtship behaviours including courting, scrape ceremony and copulation (Urban et al., 1986; Hockey et
164 al., 2005, see video of Kittlitz's plover courting behaviour in the Supplementary Material, Video S1).
165 New pairs were checked for clutches every day, and eggs were measured as described above. Two
166 observers recorded all behavioural observations (M. Beltrán and J.E. Parra).

167

168 *Molecular Sexing*

169

170 Both plover species have sexually monomorphic plumage (Hockey et al., 2005; Urban et al.,
171 1986), therefore we used molecular sex-typing to determine the sex of individuals (dos Remedios, Lee,

172 Székely, Dawson, & Küpper, 2010). A small blood sample was taken from each adult's brachial wing
173 vein, by puncturing, collecting drops of blood (~25 ul) in capillary tubes, and storing this in Eppendorf
174 tubes of Queen's Lysis Buffer. DNA was extracted from blood samples using the Ammonium Acetate
175 extraction method (Miller, Dykes, & Polesky, 1988; Richardson, Jury, Blaakmeer, Komdeur, & Burke,
176 2001). For molecular sex-typing, Z- and W-chromosome specific genes were amplified via polymerase
177 chain reaction (PCR) using the Z-002B/Z-002D primers (Dawson, 2007). For additional certainty in sex
178 assignment, the W-chromosome specific Calex-31 primers, developed in the genus *Charadrius* were
179 utilized (Küpper, Horsburgh, Dawson, Ffrench-Constant, Székely, & Burke, 2006). PCR amplification
180 was conducted on a DNA Engine Tetrad 2 Peltier Thermal Cycler under the following conditions: 95°C
181 for 15 min, followed by 35 cycles of 94°C for 30 s, 56°C for 90 s, 72°C for 60 s with a final extension of
182 60°C for 30 min. Samples were visualized on an ABI 3730 automated sequencer. IR Dye-labelled tailed
183 primers separated the products of Z-002B/Z-002D primers into either one (ZZ) or two bands (ZW),
184 indicating male or female respectively. The W-specific Calex-31 product appeared as one band indicating
185 female only. Images were scored using GeneMapper software version 4.1 (Applied Biosystems). To
186 maximize reliability, all samples were sexed using two markers. Additionally, for 8% of samples (11
187 Kittlitz's plover and 10 white-fronted plover individuals) molecular sexing was repeated; in all cases,
188 repetitions concurred with the original results.

189

190 *Data Processing and Statistical Analyses*

191

192 Date of mating was defined as the mean date between the date when a plover was last seen single
193 and the first date it was seen with a new mate. Remating time was the difference between date of release
194 (either on the day of manipulation or from captivity) and date of mating. The response variable, remating
195 time, was analyzed using generalized linear models (GLM) with Tweedie (1.5) error structure and a log
196 link function (Smyth & Verbyla, 1999). The models investigated the effects of two main variables:

197 species (Kentish, white-fronted and Kittlitz's plovers) and sex; and three additional fixed variables: type
198 of manipulation (released in the field or released from captivity), release date, and number of days in
199 captivity (see Table 1). Dates were expressed as Julian dates, i.e. number of days since 1 January. Results
200 of backward elimination based on Akaike's information criterion for small sample sizes (AICc) are
201 presented for variable selection of the GLM models where lowest AICc score is the best supported model
202 (Symonds & Moussalli, 2010; Table 1). Pairwise multiple comparisons were performed to compare mean
203 differences of mating time between males and females of the three species using Fisher's least significant
204 difference tests (LSD test).

205 Remating time was also analyzed using survival analyses and these estimates are referred to as
206 expected remating times (see rationale in Székely et al., 1999). In these analyses, the terminal event
207 (outcome) was the occurrence of mating, defined as the first observation when a plover was seen with a
208 mate. Several individuals did not find a new mate when we saw them for the last time, and these were
209 treated as censored observations. First, we used a Gehan-Wilcoxon test to compare expected remating
210 times curves (survival curves) for three species by sex. Survival curves were generated by the Kaplan-
211 Meier method. Second, for each plover species a separate Cox regression model was constructed to
212 investigate the probability of remaining single from the day of release (season), sex and their interaction
213 as covariates (Table 2).

214 Courting behaviour was estimated as percentage obtained from each 30 min sample. For
215 individuals with several behavioural observations, we calculated the mean percentage of courting.
216 Courting behaviour was analyzed using GLMs with Tweedie (1.5) error structure and a logarithmic link
217 function per individual plover. The model included two main factors: species of plover and sex; and three
218 additional fixed variables: type of manipulation (released in the field or from captivity), released date, and
219 number of days in captivity. Model selection and statistical parameters estimated for each independent
220 variable in the models are provided in the Appendix, Table A1. In addition, for each sex a separate GLM
221 model was constructed to investigate the effect of three species of plovers on courting behaviour (Table

222 3). Pairwise multiple comparisons were performed to compare mean differences of courtship behaviour
223 between males and females in the three species of plovers using LSD tests.

224 Finally, pair bond stability was analysed comparing the frequencies of mate replacement between
225 white-fronted plover and Kittlitz's plover with Fisher's exact test. Data were analyzed by using SPSS
226 statistics for Windows version 19 and figures were made in R (R Development Core Team, 2008) using
227 the package ggplot2 (Wickham, 2009).

228

229 *Ethical Note*

230

231 The experiments in Madagascar were approved by the Ministry of Environment, Forests and
232 Tourism of the Republic of Madagascar (Research permit No: 053/11/MEF/SG/DGF/DCB.SAP/SCB of
233 11 March 2011 and 132/10/MEF/SG/DGF/DCB.SAP/SSE of 06 May 2010) and Madagascar National
234 Parks (No: 398-10/MEF/SG/DGF/DVRN/SGFF of 18 May 2011). Blood sampling was also covered by
235 these research permits. The blood transport permit was approved by Service de la Gestion de la Faune et
236 de la Flore, Direction de la Valorisation des Ressources Naturelles, Ministère de l'Environnement et des
237 Forêts Madagascar (authorization number 080N-EA06/MG11). The Kentish plover experiment was
238 approved by the Turkish Ministry of Environment (see Székely et al., 1999). The Kittlitz's and the white-
239 fronted plovers are common breeding birds in much of Africa and Madagascar and they are not
240 considered threatened by the IUCN (BirdLife International, 2012). Captive plovers were monitored daily
241 and kept under standard conditions (see Experimental Manipulation) to reduce their stress levels. In
242 addition, translocated eggs coped with the natural breeding conditions of local clutches in the two plover
243 populations (see above). The experiment was designed to reduce adverse effects on plover welfare and
244 their local populations.

245

246 **RESULTS**

247

248 *Between Species Comparison*

249

250 Remating opportunities differed significantly between the three plover species (Fig. 1): white-
251 fronted plovers mated significantly more quickly (median = 2.0 days, range 0.5 – 4.5 days, $N = 12$) than
252 both Kittlitz's plovers (median = 5.1 days, range 1.0 – 11.5 days, $N = 16$) and Kentish plovers (median =
253 6.3 days, range 0.5 – 47.5 days, $N = 34$; Table 1).

254 These results remained consistent using survival analyses that also included the individuals that
255 were not successful in finding a new mate (Fig. 2, Table 2; see Methods). The proportion of plovers
256 remaining single was significantly lower for the white-fronted plover (median = 4 days, $N = 14$) than both
257 the Kittlitz's plover (median = 14.6 days, $N = 33$) and Kentish plover (median = 13.4 days, $N = 59$; testing
258 the three species, Wilcoxon–Gehan test: $\chi^2_{2} = 16.316$, $P < 0.001$).

259

260 *Between Sexes Comparison*

261

262 A significant species by sex interaction suggested a sex-biased difference in remating
263 opportunities (GLM: $\chi^2_{2} = 47.62$, $P < 0.001$, Table 1). Female Kittlitz's plovers took significantly longer
264 to mate (median = 6.5 days, range 3.5 – 11.5 days, $N = 6$) than males (median = 3.3, range 1.0 – 7.5 days,
265 $N = 10$; LSD test: pairwise mean difference = -0.66, $df = 1$, $P = 0.047$) whereas the opposite was found in
266 the Kentish plover (Székely et al. 1999). However, male and female remating times were not significantly
267 different in white-fronted plovers (male: median: 2.0 days, range 0.5 – 3.5 days, $N = 6$; female: median:
268 2.0 days, 1.0 – 4.5 days, $N = 6$, LSD test: pairwise mean difference = -0.11, $df = 1$, $P = 0.823$).

269 These results remained consistent using survival analyses (Table 2): the proportion of female
270 Kittlitz's plovers remaining single was higher than that of males (male median: 11.0 days, $N = 17$, female

271 median: 21.0 days, $N = 16$, Fig. 2), whereas the proportion of single males and females were not
272 significantly different in the white-fronted plover (male median: 3.0 days, $N = 7$, female median: 4.0 days,
273 $N = 7$, Fig. 2). Remating time increased over the season only for female Kentish plovers (Cox regression:
274 $\chi^2_1 = 7.66$, $P = 0.014$), suggesting an influence of time of breeding season on mating opportunities in the
275 Kentish plover, although this was not the case in the other two species (Table 2).

276

277 *Courtship Behaviour*

278

279 Courtship behaviour had a significant species by sex interaction (GLM: $\chi^2_2 = 6.329$, $P = 0.042$,
280 Supplementary Material: Table S2, Fig. 3). Courtship behaviour by males differed significantly between
281 species (GLM: $\chi^2_2 = 10.689$, $P = 0.005$, Table 3), male Kentish plovers spent significantly more time on
282 courtship than males of the other plover species; whereas courtship behaviour by females did not differ
283 between species (GLM: $\chi^2_2 = 1.437$, $P = 0.487$, Table 3). In contrast to the Kentish plover, which
284 exhibited male-biased courtship behaviour (LSD test: pairwise mean difference = -3.29, $df = 1$, $P =$
285 0.005), males and females of the other two species spent comparable times on courtship (LSD test white-
286 fronted plover: pairwise mean difference = 1.06, $df = 1$, $P = 0.252$; LSD test Kittlitz's plover: pairwise
287 mean difference = -0.36, $df = 1$, $P = 0.679$, Fig. 3).

288

289 *Pair Bonds*

290

291 The new pair bonds in experimentally-induced white-fronted plovers were significantly weaker
292 than in Kittlitz's plover: in 12 white-fronted plovers that remated after their former partner was removed
293 (6 males, 6 females), all experimentally-induced pair bonds were replaced by the original mates after they
294 were released from captivity. In contrast, in 16 Kittlitz's plovers that remated after their former partner

295 was removed (10 male and 6 female), none were replaced by their former mates once their former mates
296 were released from captivity (Fisher's exact test: mate replacement in white-fronted plover = 12, $N = 12$;
297 mate replacement in Kittlitz's plover = 0, $N = 16$; $P < 0.001$)

298

299 **DISCUSSION**

300

301 These experiments provided four key results. First, they show that mating opportunities are
302 significantly different between closely related species. This result is striking because two of these plover
303 species (white-fronted and Kittlitz's) breed in the same habitat in Madagascar, and therefore, ecological
304 factors are unlikely to explain the differences in remating opportunities. The rapid remating of white-
305 fronted plovers – a pattern we did not expect – suggests that there is a large pool of floating individuals
306 that can rapidly move in to pair up with unmated individuals. Consistent with the latter argument, both
307 male and female white-fronted plovers stayed in the same territory, and new individuals moved in to
308 replace the removed mates. Mating opportunities seem to be an important factor in the evolution of
309 breeding systems across a range of species (Balshine-Earn & Earn, 1998; Magrath & Komdeur, 2003).
310 For example, as in the white-fronted plover, male dunlin (*Calidris alpina*) were rapidly replaced by other
311 males after experimental removal from their breeding territories (Holmes, 1970; Pitelka, Holmes, &
312 Maclean, 1974). In the European starling (*Sturnus vulgaris*), after mating opportunities were increased by
313 the provision of additional nest-boxes, males increased their mating effort to attract additional mates and
314 also reduced parental care effort (Smith, 1995), and in St. Peter's fish (*Sarotherodon galilaeus*), males
315 and females were more likely to desert the offspring when remating opportunities were increased
316 experimentally (Balshine-Earn & Earn, 1998).

317 Second, we found sex-bias in remating opportunities: the male-biased remating opportunities in
318 Kittlitz's plover were the opposite of those found in the Kentish plover (Székely et al., 1999), whereas in
319 white-fronted plovers remating opportunities did not differ between males and females. As far as we are

320 aware, our study is the first to experimentally demonstrate differences in sex-biased remating
321 opportunities between closely related species in wild populations. Sex-biased mating opportunities may
322 emerge in two ways. One explanation is that the ratio of sexually active males to females (operational sex
323 ratio, OSR) may not be at parity. OSR may be biased due to differences in the reproductive schedules of
324 males and females (“time in”: time spent in the mating pool, sexually active; “time out”: time spent out of
325 the mating pool, not sexually active), or biased adult sex ratios (ASR; Donald, 2007). Evidence suggests
326 that OSR can vary due to mating and parental care activities (Forsgren et al., 2004, Symons, Svensson, &
327 Wong, 2011; LaBarbera, Lovette, & Llambías, 2011; Canal, Jovani, & Potti, 2012). In addition, recent
328 studies found substantial difference in ASR between closely related shorebird species (Liker et al., 2013).
329 Further works are needed to separate whether biased remating opportunities emerge via different
330 reproductive scheduling or biased ASR in plovers. For one of these species, the Kentish plover, a
331 demographic study confirmed male-biased ASR (Kosztolányi et al., 2011), although ASR has not been
332 estimated for the white-fronted and the Kittlitz’s plover. Alternatively, sex-biased mating opportunities
333 may arise due to differences in the willingness of males and females to remate. For instance, the post-
334 breeding refractory periods, the recovery phase spent preparing for another breeding attempt, may differ
335 between males and females (Balshine-Earn & Earn, 1998; Cantoni & Brown, 1997): females typically
336 need more time to recover than do males. However, the latter explanation is unlikely, since the adult
337 plovers used in our experiments had breeding efforts interrupted and sought new mates shortly after
338 removal of their mate (or on release from captivity). Furthermore, several female Kentish plovers remated
339 within less than a day – a pattern that is inconsistent with the explanation that females need more time to
340 recover than do males.

341 Third, male courtship behaviour was different between the three species, since male Kentish
342 plovers spent more time on courtship than male white-fronted and Kittlitz’s plovers. This pattern is
343 consistent with the explanation that ASR is male-biased in the Kentish plover. The significance of this
344 result is that courtship behaviour is variable between closely related species and suggests that ASR, and in

345 turn the OSR, is probably related to the intensity of mating competition in males. Consequently,
346 comparable intensity of courtship behaviour was observed between sexes in the Kittlitz's and white-
347 fronted plovers. The latter result indicates that males and females may compete similarly for available
348 mates, suggesting that they do not have conventional sex roles: male-male competition and female choice
349 for mates (Vincent, Ahnesjö, & Berglund, 1994). Variation in resources for breeding has also been
350 suggested to influence OSR, and in turn, the intensity of mating competition (Forsgren, Kvarnemo, &
351 Lindstrom, 1996). Availability of breeding territories, for example, may affect the OSR, since the sex that
352 holds the territories will be limited by scarcity of nest sites. In a sand goby population (*Pomatoschistus*
353 *minutes*), for instance, nest-site abundance can influence the intensity of male mating competition
354 (Forsgren et al., 1996). Hence, the dynamic of OSR, and in turn mating competition, is probably
355 modulated by both ASR and resource availability (Kvarnemo & Ahnesjö, 1996, Forsgren et al., 2004).

356 Finally, the new pair bonds were significantly weaker in white-fronted plovers than in Kittlitz's
357 plover suggesting that the former species exhibits long-term pair bonds whereas the latter has short-term
358 pair bonds. Mate fidelity may emerge in two ways. On the one hand, former mates may actively seek out
359 each other *per se*, and prefer to mate with each other. On the other hand, mate fidelity may emerge via
360 site fidelity: white-fronted plovers are highly territorial (Lloyd, 2008), and therefore upon release from
361 captivity, individuals return to their former territories and chase out their former partner's new mates.
362 Established pairs may prefer to reunite because of the fitness benefits in terms of synchronisation of
363 behavioural and physiological characteristics such as defence of breeding territories, courtship
364 behaviours, laying date, incubation, chick-raising between others which have been shown to improve with
365 time and experience of the pair (Bried, Pontier, & Jouventin, 2003; Rowley, 1983). An experiment carried
366 out in bearded reedling *Panurus biarmicus* found that long-term pair bond formation improved
367 coordination of breeding activities and reproductive success (Griggio & Hoi, 2011). In another example,
368 newly formed pairs (either due to divorce or loss of a mate) had lower reproductive success than
369 established pairs in black turnstone *Arenaria melanocephala* (Handel & Gill, 2000). Improved breeding

370 with increased experience is also well known in geese, albatrosses and other long-term pair bonding
371 animals (Angelier, Weimerskirch, Dano, & Chastel, 2006; Black, 2001). Overall, the consequences of
372 pair bond and site fidelity on mating opportunities could be significant since the ability of an individual to
373 mate may be limited by their access to mates and breeding sites.

374 In conclusion, using an experimental approach we found significant differences in remating
375 opportunities between closely related plover species. As mating opportunity is linked to OSR and ASR,
376 our work suggests that substantial variation in OSR (and possibly ASR) is exhibited among closely
377 related species. Such variation may influence the direction and intensity of competition in males and
378 females for mates and breeding territories. These differences in OSR, in turn, may facilitate different
379 intensities of sexual selection and induce different mating systems and patterns of parental care.

380

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529 **Figure Legends**

530

531 **Figure 1.** Remating times in three plover species. The lower and upper borders of the box are lower and
532 upper quartiles, respectively, the horizontal bar is the median and whiskers represent the lowest and
533 highest observations.

534

535 **Figure 2.** Proportion of males and females remaining single in three plover species: Kentish plover (top),
536 white-fronted plover (middle) and Kittlitz's plover (bottom). Dotted lines show the expected mating time
537 of males and females after release. Number of individuals: 32 male and 27 female Kentish plovers; 7 male
538 and 7 female white-fronted plovers; and 17 male and 16 female Kittlitz's plovers.

539

540 **Figure 3.** Courtship behaviour in three plover species. The lower and upper borders of the box are lower
541 and upper quartiles, respectively, the horizontal bar is the median and whiskers represent the lowest and
542 highest observations. Circles denote outliers that are between the first and third interquartile from the
543 nearer edge of the box.

544 **Table 1.**

545 Remating times (response variable) of males and females in three species of plover.

Independent variable	Full model (AICc = 346.004)			Best model (AICc = 341.088)		
	Wald χ^2	Df	P	Wald χ^2	Df	P
(Intercept)	38.596	1	<0.001	49.365	1	<0.001
<i>Between species comparison</i>						
Species	11.248	2	0.004	11.595	2	0.003
<i>Between sexes comparison</i>						
Sex	4.072	1	0.044	3.974	1	0.046
Species * sex	39.65	2	<0.001	47.620	2	<0.001
Manipulation	0.290	1	0.59	-	-	-
Release date	4.818	1	0.028	5.007	1	0.025
Captive days	0.646	1	0.422	-	-	-

546 GLMs were used to analyze mating time using Tweedie (1.5) error structure and a log link function.

547 Model selection was carried out using Akaike information criterion for small sample sizes (AICc).

548 Manipulation refers to whether a plover was kept in captivity or not. Release date refers to the date when
549 a plover was released to find a new mate (Julian dates). Captive days are the number of days that a plover
550 was kept in captivity.

551

552 **Table 2.**

553 Remating time in plovers based on Cox proportional hazard models, to examine the relationship of the
 554 survival distribution which includes censored observations.

Species	Variable	B	Wald χ^2	df	p
Kentish plover	Sex	1.541	12.07	1	0.001
	Release date	-0.024	6.073	1	0.014
White-fronted plover	Sex	-0.18	0.083	1	0.77
	Release date	0.002	0.004	1	0.95
Kittlitz's plover	Sex	-1.342	4.864	1	0.027
	Release date	-0.01	0.088	1	0.767

555 For each species a separate model was constructed. Number of individual Kentish plovers, mated = 34,
 556 censored = 19; white-fronted plover, 12, 2; Kittlitz's plover, 16, 17, respectively.

557

558 **Table 3.**

559 Courtship behaviour (response variable: % of time courting) in three plover species.

Sex	Variables	Wald χ^2	<i>Df</i>	<i>P</i>
Male	(Intercept)	13.176	1	< 0.001
	Species	10.689	2	0.005
Female	(Intercept)	0.155	1	0.694
	Species	1.437	2	0.487

560 GLMs were used to analyse percentage of time courting using Tweedie (1.5) error structure and a log link

561 function. Model selection was carried out using Akaike information criterion for small sample sizes

562 (AICc).

563 **APPENDIX**

564 **Table A1.**

565 Courtship behaviour in plovers (response variable: proportion of time courting).

Independent variable	Full model (AICc = 269.035)			Best model (AICc = 261.316)		
	Wald χ^2	d.f.	p	Wald χ^2	d.f.	P
(Intercept)	2.215	1	0.137	2.914	1	0.088
Species	0.621	2	0.733	0.791	2	0.673
Sex	5.713	1	0.017	5.381	1	0.020
Species * sex	6.084	2	0.048	6.329	2	0.042
Manipulation	0.057	1	0.811	-	-	-
Release date	0.056	1	0.813	-	-	-
Captive days	0.477	1	0.490	-	-	-

566 GLMs were used to analyse courtship behaviour using Tweedie (1.5) error structure and a log link
 567 function. Model selection was carried out using Akaike information criterion for small sample sizes
 568 (AICc).

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2

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Figure 1

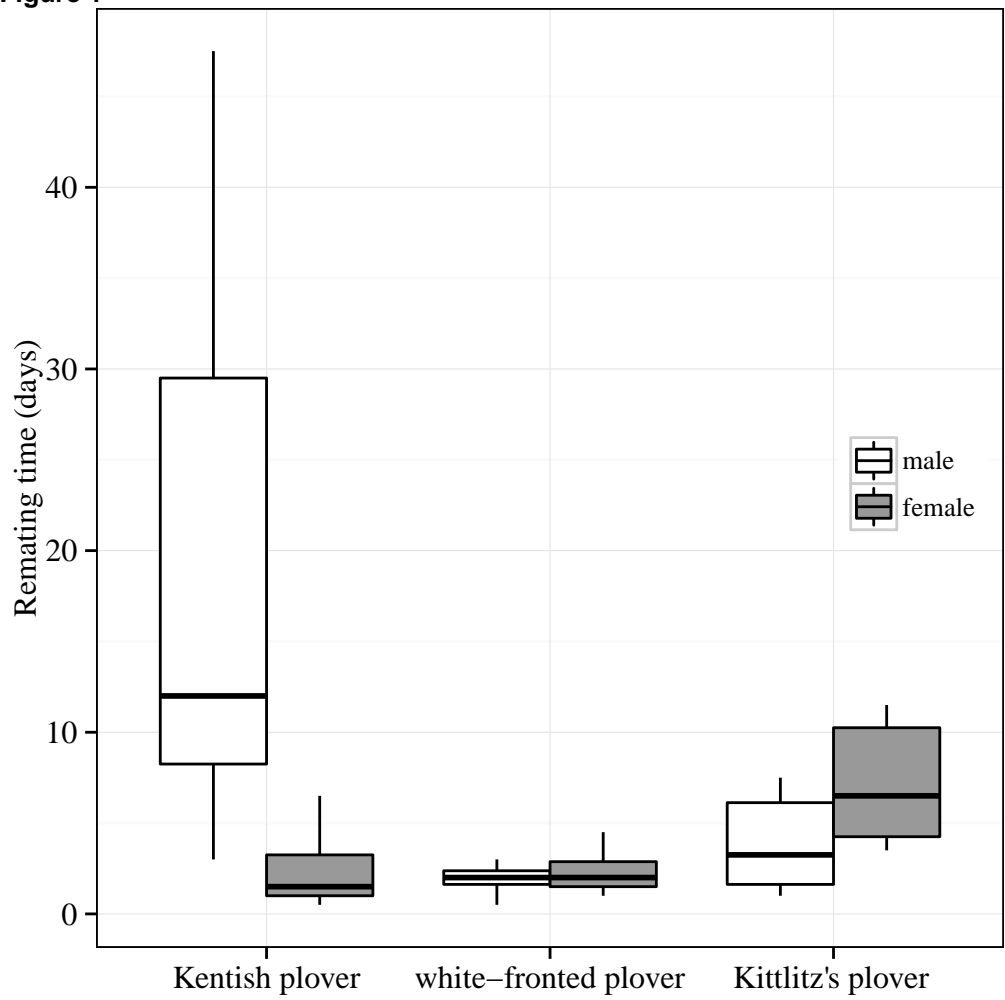


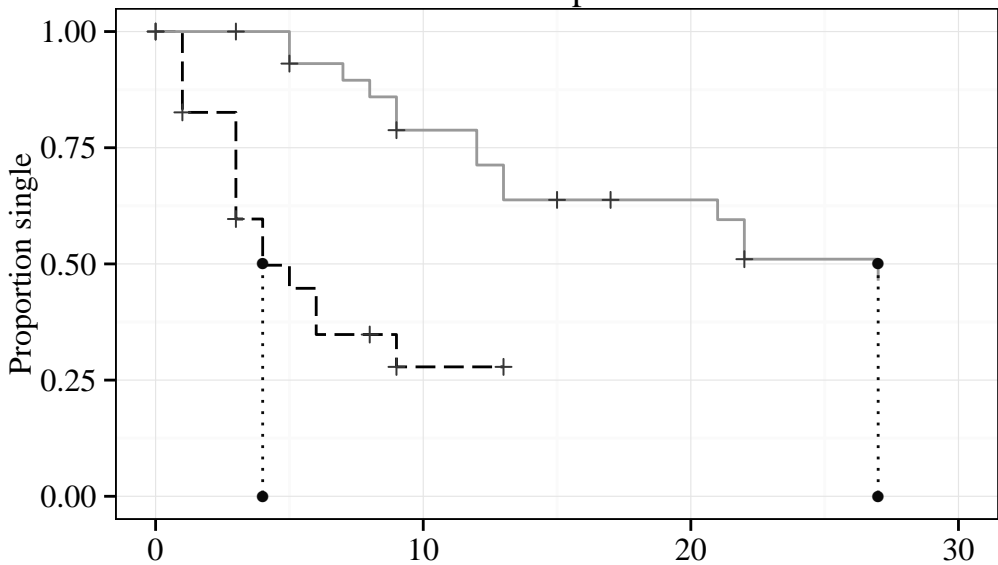
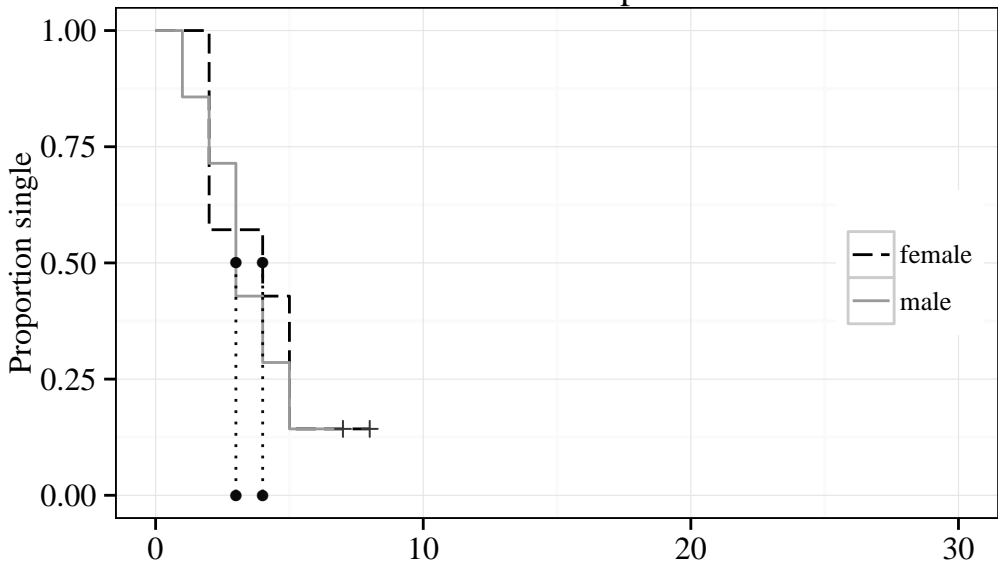
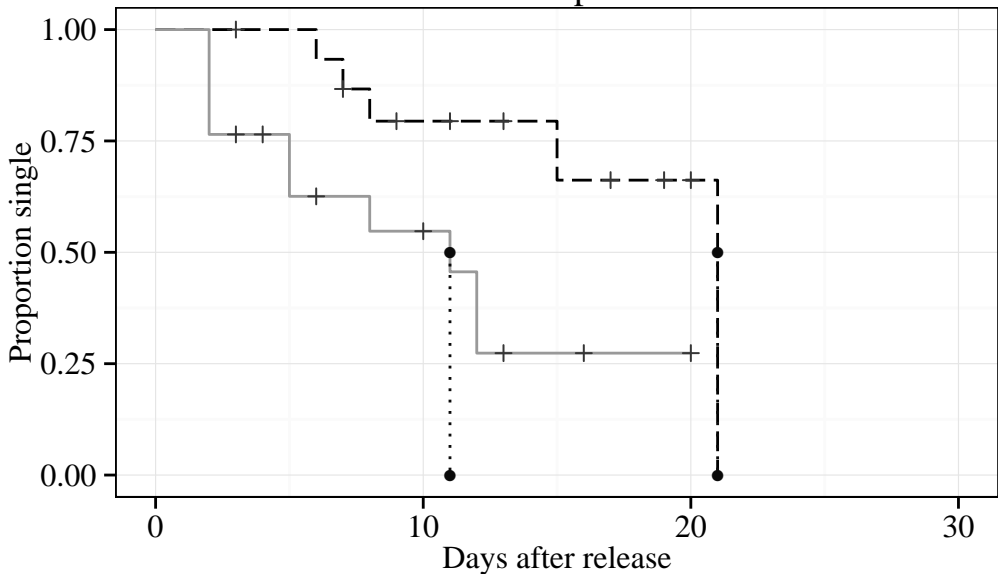
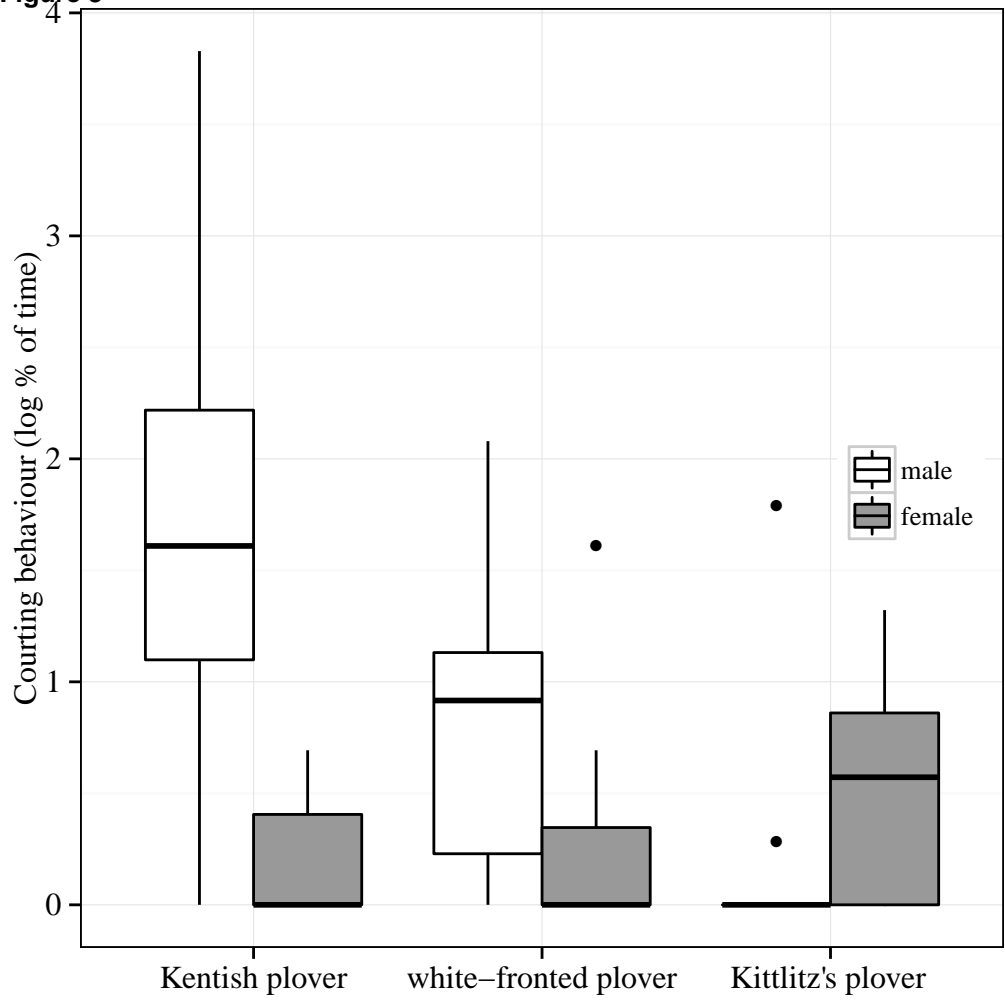
Figure 2**Kentish plover****white-fronted plover****Kittlitz's plover**

Figure 3



Video S1
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Caption Video S1

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