The Auk 123(2):524–536, 2006
© The American Ornithologists' Union, 2006.
Printed in USA.

MATE OPPORTUNITY HYPOTHESIS AND EXTRAPAIR PATERNITY IN WAVED ALBATROSSES (*PHOEBASTRIA IRRORATA*)

Kathryn P. Huyvaert,[1,3] David J. Anderson,[2] and Patricia G. Parker[1]

[1]*Department of Biology, University of Missouri-St. Louis, 8001 Natural Bridge Road, St. Louis, Missouri 63121, USA; and*
[2]*Department of Biology, Wake Forest University, Winston-Salem, North Carolina 27109, USA*

Abstract.—Early arrival at breeding sites can influence reproductive success through enhanced access to critical resources such as nest sites or mates. One hypothesis explaining protandry, or male-first arrival at breeding sites, proposes that males arrive earlier to increase their extrapair copulation (EPC) opportunities, which may, in turn, enhance males' reproductive success through extrapair fertilizations (EPFs) ("mate opportunity hypothesis"). Extrapair behavior is unexpected in long-lived birds, in which the male is expected to abandon a brood of uncertain paternity, because his probability of future reproduction is high. A previous study of the Waved Albatross (*Phoebastria irrorata*), a long-lived, socially monogamous seabird, showed evidence of EPFs in 4 of 16 (25%) families. Here, we combined behavioral observations of copulations with additional molecular genetic evidence of EPFs in Waved Albatross families to investigate the fitness consequences of protandry under the mate opportunity hypothesis. During three breeding seasons, we documented 3,661 attempted copulations between birds of known identity; >60% of copulations that involved at least one breeding bird were classified as EPCs. Protandry was pronounced in all three study years: 76.3–96.6% of males arrived before (typically, 6–10 days before) their social mates. Early arrival was associated with increased opportunities for copulations: individual EPC frequencies were higher for breeding males than for breeding females, and males that arrived earlier than their social mates engaged in the most EPCs. Extrapair fertilizations were also regular in our study population; social fathers were excluded as the genetic sire in 14–21% of families. Egg laying dates of EPFs tended to be earlier in the season than those of within-pair fertilizations (WPFs). However, earlier arrival by a male did not translate into a higher probability of siring either within-pair or extrapair offspring, nor did cuckolding males have enhanced reproductive success. Although the fitness consequences of early arrival remain unclear, these findings suggest alternative advantages of early arrival for male Waved Albatrosses. *Received 27 September 2004, accepted 16 August 2005.*

Key words: extrapair copulations, extrapair paternity, mate opportunity hypothesis, *Phoebastria irrorata*, protandry, Waved Albatross.

La Hipótesis de Oportunidad de Apareamiento y Paternidad Extra-pareja en *Phoebastria irrorata*

Resumen.—La llegada temprana a los sitios de apareamiento puede influenciar el éxito reproductivo mediante el acceso privilegiado a los recursos críticos como los sitios de nidificación o las parejas. Una hipótesis que explica la protandria, o

---

[3] Present address: Department of Fishery and Wildlife Biology, Colorado State University, Fort Collins, Colorado 80523, USA. E-mail: huyvaert@cnr.colostate.edu

la llegada anticipada de los machos a los sitios de apareamiento, propone que los machos llegan primero para incrementar sus oportunidades de copulación extra-pareja (CEP), lo que puede, a su vez, incrementar el éxito reproductivo de los machos mediante las fertilizaciones extra-pareja (FEP) ("hipótesis de oportunidad de apareamiento"). El comportamiento extra-pareja es inesperado en las aves longevas, en las cuales se espera que los machos abandonen las nidadas cuya paternidad sea incierta, debido a que la probabilidad de un futuro evento reproductivo es elevada. Un estudio previo del albatros *Phoebastria irrorata*, un ave marina longeva socialmente monógama, mostró evidencia de FEP en 4 de 16 (25%) familias. Aquí, combinamos observaciones de comportamiento de cópula con evidencias genéticas moleculares adicionales de FEP en las familias de *P. irrorata* para investigar las consecuencias de la protandria sobre la adecuación biológica en el marco de la hipótesis de oportunidad de apareamiento. Durante tres estaciones reproductivas, documentamos 3,661 intentos de copulación entre aves de identidad conocida. Más del 60% de las cópulas que incluyeron al menos un ave reproductiva fueron clasificadas como CEP. La protandria fue elevada en las tres áreas de estudio: 76.3–96.6% de los machos llegaron antes (típicamente, 6–10 días antes) que sus parejas sociales. La llegada temprana se asoció con un incremento en las oportunidades de copulación: las frecuencias individuales de CEP fueron mayores para los machos reproductivos que para las hembras reproductivas, y los machos que llegaron más temprano que sus parejas sociales participaron en la mayoría de las CEP. Las fertilizaciones extra-pareja también fueron frecuentes en nuestra población de estudio; los padres sociales fueron excluidos como los procreadores genéticos en el 14–21% de las familias. Las fechas de puesta de los huevos de las FEP tendieron a darse más temprano en la estación que las fertilizaciones intra-pareja. Sin embrago, la llegada temprana de un macho no se tradujo en una mayor probabilidad de engendrar pichones tanto intra- como extra-pareja, ni tampoco los machos cornudos tuvieron un éxito reproductivo mayor. Aunque las consecuencias para la adecuación biológica de la llegada temprana permanecen poco claras, estos resultados sugieren ventajas alternativas de la llegada temprana de los machos de *P. irrorata*.

Demographic factors, including a breeding population's density and operational sex ratio, are believed to influence whether male birds encounter extrapair mating opportunities by affecting the availability and dispersion of potential mates (Birkhead and Møller 1992). In turn, temporal factors such as breeding synchrony and arrival timing may influence reproductive success (e.g. Stutchbury 1998) if, for example, extrapair copulations (EPCs) are timed to coincide with female fertile periods and are thus more likely to lead to fertilizations. Earlier arrival at breeding sites (or earlier emergence timing by males), termed "protandry," characterizes the breeding phenology of many organisms, including many migratory bird species. Here, we employ the term in this sense, though it has widely been used to describe male-first patterns of sexual function in taxa that are hermaphroditic, sex-changing, or monoecious (e.g. Charnov 1982). In their comprehensive review, Morbey and Ydenberg (2001) grouped hypotheses explaining protandrous arrival in animals by asking whether selection operates on timing within each sex independently (indirect hypotheses) or on the relative timing between the sexes (direct hypotheses). These authors argue that, among hypotheses explaining protandry in birds, strongest support exists for the indirect "rank advantage" hypothesis advanced by Ketterson and Nolan (1976) for territorial species, wherein competition for the best territories drives early arrival by the sex that chooses the pair's territory, typically the male.

By contrast, data on Red-necked and Wilson's phalaropes (*Phalaropus lobatus* and *P. tricolor*) support the direct "mate opportunity" hypothesis (Reynolds et al. 1986). Because neither sex maintains a territory in these species, female-first arrival is most likely a result of sexual selection operating on females to arrive earlier than males to acquire the most mating opportunities. In the case of protandry, the mate opportunity hypothesis proposes that earlier-arriving males have better success encountering mates, which ultimately enhances their reproductive success. In socially monogamous birds, fitness can be enhanced through reproductive success via both within-pair copulations (WPCs) and EPCs, assuming that EPCs can result in extrapair fertilizations (EPFs). Early arrival pays, if it increases the probability of encountering arriving females, simply by increasing the number of encounter opportunities. Early arrival may also increase encounter probability by increasing the time a male is free from mate-guarding by or of his social mate (the female with whom he shares incubation or caretaking duties, or both, of the same egg or chick). Thus, if the mate opportunity hypothesis applies to a socially monogamous bird species, it specifically predicts the following: (1) males arriving earlier than their social mates will have more EPCs, (2) males arriving earlier than their social mates will be more likely to sire extrapair young (EPY), and (3) males siring EPY will have higher overall reproductive success.

Older models of avian mating systems assumed that observed copulation behavior reflected genetic patterns (Emlen and Oring 1977), but the advent of genetic techniques revealed an array of avian social behavior, including widespread occurrence of EPCs that result in EPFs in socially monogamous taxa (see Griffith et al. [2002] and Westneat and Stewart [2003] for reviews). Nevertheless, although levels of extrapair paternity as high as 72% have been reported for passerine taxa such as the Superb Fairy-Wren (*Malurus cyaneus*; Dunn and Cockburn 1998), theoretical and empirical studies continued to predict and to demonstrate relatively low EPF rates in long-lived nonpasserines with essential male care (e.g. Mauck et al. 1999, Schwartz et al. 1999, Dearborn et al. 2001). Indeed, biparental care is essential, and lifespans can reach ≥40 years in the procellariiforms ("tubenosed" seabirds, including petrels, shearwaters, and albatrosses); accordingly, most EPF rates are typically low, ranging from zero in Northern Fulmars (*Fulmarus glacialis*; Hunter et al. 1992) and Leach's Storm Petrel (*Oceanodroma leucorhoa*; Mauck et al. 1995) to 9–13% in Short-tailed Shearwaters (*Puffinus tenuirostris*; Austin and Parkin 1996). We thus expected the frequency of EPFs in the Waved Albatross (*Phoebastria irrorata*) to be low.

After a two-month absence at sea, adult male Waved Albatrosses return to the colony at the beginning of each breeding season, typically in mid-March, and adopt territorial behavior; they are followed later by returning adult females (Harris 1973, present study). Egg-moving during incubation is common in Waved Albatrosses (Awkerman et al. 2005). Mobile incubation means that territories can be fluid, though males typically return to within a few meters of the starting position of previous nesting sites and, except for short foraging trips and occasional walks about the colony, remain at the site until their social mates return and nesting ensues (K. Huyvaert et al. unpubl. data). As other birds return to the colony, male Waved Albatrosses aggressively chase away approaching birds of both sexes, and these encounters occasionally end in apparent EPCs that may be associated with territoriality (Harris 1973). Tickell's (1968) study of the Wandering Albatross (*Diomedea exulans*) raised the point that another possible function of this type of copulation is enhanced reproductive success through EPFs. Indeed, some males that are waiting for their mates to arrive engage in apparently nonaggressive copulations with neighboring females or females they encounter while moving about the colony (K. Huyvaert et al. unpubl. data). In a preliminary study, we used multilocus minisatellite DNA fingerprinting to estimate the rate of EPFs in the Waved Albatross. Analyses of 16 families revealed evidence of EPFs in 4, though the true EPF rate, based on binomial confidence intervals for this estimate of 25%, could be as low as 7% or as high as 52% (Huyvaert et al. 2000). Here, we combine three seasons of behavioral observations with molecular assessments of parentage to obtain a better estimate of the proportion of families affected by EPF and to investigate the fitness consequences of protandry by testing the predictions of the mate opportunity hypothesis.

Methods

Study site and banding.—We recorded arrival times of males and the frequency and genetic consequences of EPC behavior during the breeding seasons of 2000, 2001, and 2002. Our study took place on an oblong ~3,500-m^2 area, divided into 10-m^2 quadrats and adjacent buffer zones, 2–10 m wide (~300 m^2 total), along the coast at Punta Cevallos on the southeastern end of Española, Galápagos, Ecuador (1°S, 89°W). With the exception of a few pairs on Isla de La Plata off the coast of Ecuador, Española is the main breeding site for this species (Harris 1973, Anderson et al. 2002). Adults are present and breeding each year between approximately April and December, and are absent during the intervening nonbreeding period (Harris 1973, K. Huyvaert pers. obs.).

To reduce disturbance in the colony during the study beginning in March 2000, we captured and banded all adult Waved Albatrosses present on the study area in May, August, and November 1999 ($n = 350$) and obtained a small blood sample (100 μL) from each adult via brachial venipuncture. We placed field-readable plastic bands with unique three-digit numbers on one leg (Pro-Touch Engraving, Saskatoon, Saskatchewan) and numbered aluminum bands (U.S. Geological Survey Bird Banding Laboratory, Laurel, Maryland) on the other leg of each bird. Additional unmarked birds arriving for the first time during the study seasons ($n = 163$) were similarly banded and sampled, and disturbance was minimized by banding these individuals when activity levels were low. Blood samples were stored in Longmire's solution, a lysis buffer (Longmire et al. 1988), and held at ambient temperature for later extraction and analysis.

Arrival timing and copulation behavior.—We conducted nightly censuses of the study area and adjacent buffer areas beginning on the day of the first Waved Albatross sighting in the colony each year (mid-March to early April). Individual band numbers and the approximate locations of all birds present in the study area were recorded between 1810 and 1830 hours every evening through the end of copulation observations (mid- to late May). These data were used to determine the date of first arrival for each bird. Copulation typically begins within a week of the first bird's return, usually mid-March each year, lessening as egg laying ends. We began observations of copulations with the return of the first 8–11 banded individuals and continued for 36–40 days each season. In 2000, the first banded bird arrived on 24 March; we conducted observations from 31 March to 10 May in that year. First arrivals were later in 2001 and 2002 (see below). Observations were conducted from 7 April to 16 May in 2001 and from 19 April to 24 May in 2002. In all three years, observations took place until 75–91% of all eggs on the study area and buffer areas had been laid. Within three days after the last observation day in each year, females had laid eggs at 85–95% of study nests.

We completed ~2,195 person-hours of behavioral observations over the three seasons. Typically, each day, two observers divided the study area and recorded behavior from ~0530 hours (first light) until 0900 hours, followed by one observer until 1500 hours (because activity levels were low), then two observers until ~1800 hours (dusk). Copulation behavior was characterized using an "all-occurrences" recording method (Altmann 1974, Martin and Bateson 1993) to document all detectable daytime instances of attempted WPC or EPC between adults on the study area. Observers scanned the study area for activity, keeping ongoing written narratives of interactions among individuals. Observers approached to within 3 m while recording interactions, but used binoculars in cases in which their approaches might have disturbed neighboring birds. Numbered driftwood markers in the colony in the corner of each 10-m^2 quadrat were used to identify locations during observations. Records included the identities of the participants, context, location, timing, and nature of each attempted copulation, as well as data on attempted mounts and mounts that did not lead to attempted copulations. An interaction qualified as an attempted copulation if the upper participant placed both feet on the lower bird's back and moved its tail from side to side ("mount–wiggle"). "Completed" copulations were those during which ejaculate was observed being placed on and drawn into the female's cloaca. To minimize variation among years and within cohorts of observers, one of us (K.P.H.) participated in observations during all three seasons and trained new observers at the beginning of each season.

Assigning sexes and determining social-pair status.—We assigned sexes for analyses presented here using a polymerase chain reaction (PCR)-based molecular sexing technique (Fridolfsson and Ellegren 1999). Breeding individuals and social mates were identified using nest-history data. We compiled nest histories by visiting every incubating bird and abandoned egg in the study area and adjacent buffer areas every one to three days in 2000, and every day in 2001 and 2002. At each nest check, we recorded the incubating bird's band number and the disposition of the egg or chick (intact, crushed, abandoned, alive, or dead). Any bird recorded as having incubated an egg was considered a breeder. In 2000, a male attending an egg post-laying was assumed to be a female's social mate if the egg had not been abandoned during any check between the time when the female had last been seen incubating and the first stint of incubation by the male, to control for the possibility of adoption of abandoned eggs by adults outside the family (Awkerman et al. 2005). In 2001 and 2002, we also noted whether we had seen members of the social pair at the site together just before laying or as they switched incubation duties, to determine social mate status. Copulations occurring between social mates were classified as WPCs. All other copulation attempts that included at least one individual that had a social mate were classified as EPCs. For analyses reported here, we excluded data for any bird seen incubating an egg but for whom no social mate was identified.

Paternity exclusion analyses.—Small blood samples (<100 uL) were collected from 2- to 6-day-old chicks via brachial or tarsal venipuncture. We also collected blood or brain-tissue samples opportunistically from unhatched chicks only after the egg had been opened by Hood Mockingbirds (*Nesomimus macdonaldi*) or abandoned for ≥10 days. Samples of both types were stored in lysis buffer in the field for subsequent extraction and analysis. We used multilocus minisatellite DNA fingerprinting to analyze the genetic parentage of offspring samples (Jeffreys et al. 1985a, b) following protocols detailed extensively elsewhere (Huyvaert et al. 2000, Huyvaert 2004). Briefly, we digested 4 μg whole genomic DNA with an excess of the restriction endonuclease *Hae*III and separated fragments on agarose gels using electrophoresis. Fragments were transferred to nylon membranes via Southern blotting and were hybridized to Jeffreys' probe 33.15 (Jeffreys et al. 1985a, b). Samples were arranged on gels in family groups, with putative parents flanking their chick's sample to minimize scoring errors attributable to intersample distance (Piper and Rabenold 1992).

We assessed the parentage of each chick or embryo by evaluating whether all offspring bands could be accounted for in the fingerprints of the putative parents (unattributable-bands analysis) and by estimating offspring genetic similarity with each putative parent as the proportion of fingerprinting bands shared (band-sharing analysis). Two individuals shared an identical band if the bands were of comparable mobility and intensity (Rabenold et al. 1990). Band-sharing coefficients were calculated as $2N_{AB}/(2N_{AB} + N_A + N_B)$, where N_{AB} is the number of bands shared by members in a dyad, N_A is the number of bands unique to individual A, and N_B is the number of bands unique to B (Wetton et al. 1987, Lynch 1990). We then evaluated the degree of overlap of the distributions of band-sharing values for first-order relatives (n = 98 mother–offspring pairs, with zero unattributable bands and no replicate mothers) compared with unrelated individuals (n = 118 chicks matched with randomly chosen, unrelated adults).

To determine extrapair parentage, we first calculated the upper 95% confidence limit (upper limit = mean + 1.96 * SD) of the distribution of band-sharing coefficients for unrelated individuals (Piper and Rabenold 1992), and set this as a cutoff band-sharing value. The distribution of unattributable bands among known first-order relatives was used to calculate the Poisson probability that novel bands were attributable to mutation alone (Rabenold et al. 1990) and to develop a criterion for parental exclusion based on unattributable bands. Thus, we classified as extrapair those offspring–parent dyads with two or more unattributable bands and with band-sharing values below 0.424 (see below).

Finally, we estimated the degree of error in assigning parentage by calculating X^p, the probability of misassigning an unrelated bird as father and s^p, the probability of misassigning an uncle as father (Rabenold et al. 1991). Additional details on the use of these methods in Waved Albatrosses are given elsewhere (Huyvaert et al. 2000, Huyvaert 2004).

Fitness consequences.—To evaluate the fitness consequences of protandry, we attempted to identify the actual sires of extrapair offspring via assignment gels that included the chick, mother, social father, and all putative fathers on the same gel (Rabenold et al. 1991). We assumed that the set of putative fathers comprised all the female's known copulation partners and males nesting within 10 m of the nest. Males were assigned as genetic fathers in cases when two criteria were met: (1) all, or all but one, offspring bands were attributed to either the mother or putative father; and (2) measures of band-sharing between chicks and each parent were equal to or greater than the upper 95% confidence limit of the distribution of band-sharing coefficients for unrelated dyads, which is 0.424 (Piper and Rabenold 1992; see below).

Nest histories were collected until late January 2003 for chicks hatching in 2002, showing which chicks fledged and died and when during that season. To estimate fledging success in 2000 and 2001, we returned to the colony immediately prior to fledging (November and December) to sample and band all chicks present within 25 m or in the study area. We later matched these near-fledging samples with hatchling samples using our multilocus minisatellite DNA fingerprinting protocol. We used chick and near-fledgling sexes and caretaking-parent identities to make putative pairs. Fledglings were then identified when the chick and near-fledgling fingerprint banding patterns matched exactly.

Data analysis.—Arrival timing and copulation frequency data were analyzed using STATISTICA software for Windows (Statsoft, Tulsa, Oklahoma). Normality was assessed using Shapiro-Wilk's W test (Zar 1999), and we used nonparametric tests of significance when the assumption was not satisfied. Unless specified, two-tailed probabilities are reported and descriptive statistics are expressed as the mean ± SD for normally distributed data or as median and range values for non-normal distributions.

Results

Arrival timing.—Male breeding Waved Albatrosses arrived earlier than breeding females in all three study seasons (Kolmogorov-Smirnov test: $P < 0.001$ in all years; Fig. 1). Breeding males arrived before their social mates in 86.4%, 96.6%, and 76.3% of cases in 2000, 2001, and 2002, respectively. Breeding males typically arrived 6–10 days before their social mates ($\text{median}_{2000} = 11$ days, range: 27 days before to 15 days after; $\text{median}_{2001} = 9$ days, range: 31 days before to 1 day after; $\text{median}_{2002} = 6$ days, range: 21 days before to 11 days after). Because the number of days males arrived before their social mates differed among the breeding seasons (Kruskal-Wallis test: $H = 21.62$, df = 2, $P < 0.0001$, $n = 230$) and mean male arrival date differed from year to year (Kruskal-Wallis test: $H = 83.91$, df = 2, $P < 0.0001$, $n = 282$; $\text{mean}_{2000} = 104.33 \pm 8.39$, $\text{mean}_{2001} = 109.00 \pm 8.70$, $\text{mean}_{2002} = 117.25 \pm 8.00$), we analyzed arrival data from the three seasons separately.

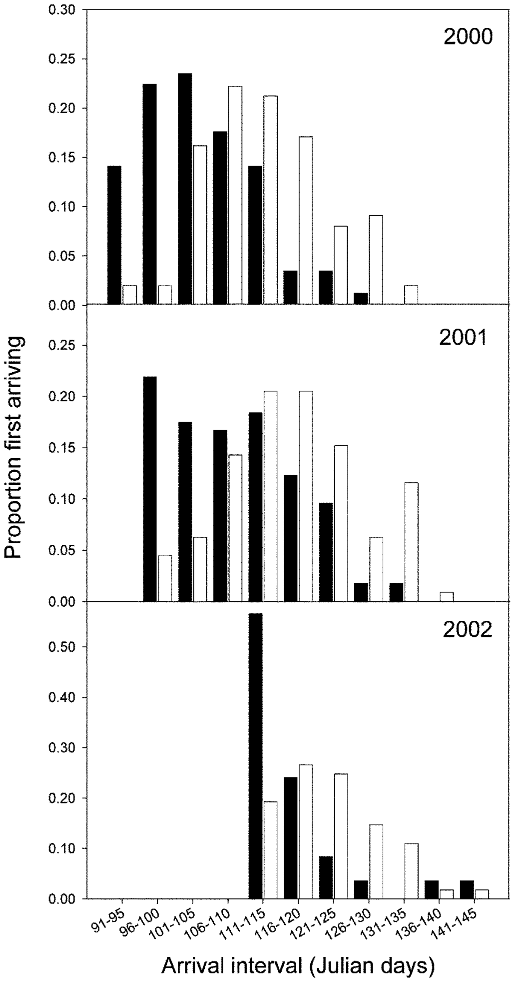

FIG. 1. Protandry in the Waved Albatross (*Phoebastria irrorata*) during three breeding seasons. Bars (filled bars, males; open bars, females) are the proportion of the total breeding individuals of each sex first seen in each five-day interval.

Extrapair copulations and protandry.—We recorded 4,854 attempted copulations; the identity of both partners was known for 3,661 of these ($n_{2000} = 1{,}281$, $n_{2001} = 1{,}167$, $n_{2002} = 1{,}213$). Of the copulations involving two birds of known identity, 59.2% (2,168 of 3,661) included a breeding female and 74.5% (2,727 of 3,661) included a

Table 1. Summary statistics for the number of extrapair copulations by breeding female and male Waved Albatrosses (*Phoebastria irrorata*) during three breeding seasons.

	Females				Males				Mann-Whitney	
Year	*n*	Mean	Median	Range	*n*	Mean	Median	Range	*U*	*P*
2000	74	3.46	1.5	0–31	74	9.00	8.0	0–27	1,197	<0.05
2001	97	2.99	2.0	0–21	97	7.45	6.0	0–35	2,654	<0.05
2002	82	2.88	2.0	0–17	82	6.93	6.0	0–24	1,852	<0.05

breeding male. Extrapair copulations accounted for 63.6% (1,379 of 2,168) of copulations by breeding females, whereas 71.7% (1,957 of 2,727) of copulations by breeding males were EPCs. Breeding females participated in an average of 2.9 to 3.5 EPCs over the three years, with a range from 0 to 31 EPCs per female and median values from 1.5 to 2.0 (Table 1). Further, 36–42% of EPCs (2000: 36.4%, 2001: 41.9%, 2002: 39.0%) by breeding females were classified as complete (i.e. ejaculate was placed on and drawn into the female's cloaca). Median EPC rates were higher for breeding males than for breeding females in all years (Table 1). Breeding males participated in an average of 6.93–9.00 EPCs and as many as 35 in one year (Table 1).

In all years, the duration of a male's presence before his social mate arrived was positively correlated with the number of EPCs that he achieved (Table 2). However, a male's Julian arrival date was not correlated with the number of his EPCs (Table 2). In two of the three years, a female's arrival date in relation to that of her social mate was negatively correlated with her number of EPCs: females arriving many days after their social mates had fewer EPCs than females arriving before or a few days after their mates. These correlations reached statistical significance in 2000 and 2001, but not in 2002 (Table 2).

Table 2. Spearman's rank-order correlations between Waved Albatross EPC numbers and protandry measured as (1) the difference in arrival in days between males and their social mates, (2) male arrival date, and (3) the difference in arrival in days between females and their social mates. Negative correlation coefficients (r_S) in (1) indicate that males typically arrived before females.

Year	*n*	r_S	*t*	*P*
(1) Males compared with social mate				
2000	66	–0.489	–4.48	<0.050
2001	88	–0.406	–4.12	<0.050
2002	76	–0.469	–4.56	<0.050
(2) Males compared with other males				
2000	73	–0.147	–1.25	0.215
2001	97	0.007	0.06	0.949
2002	80	–0.271	–2.48	0.015
(3) Females compared with social mate				
2000	66	–0.317	–2.67	<0.050
2001	88	–0.305	–2.97	<0.050
2002	76	–0.207	–1.82	0.072

Timing of copulations.—Proportions of females with each type of copulation (EPCs vs. WPCs) were not distributed differently in time in relation to egg laying during any single year (chi-square test: $\chi^2_{2000} = 0.066$, $\chi^2_{2001} = 0.087$, $\chi^2_{2002} = 0.183$, df = 15; $P = \sim 1.00$ in all years) nor for all years combined (chi-square test: $\chi^2 = 0.102$, df = 15, $P = \sim 1.00$; Fig. 2), thus meeting the assumption that EPCs occur when females are believed to be fertilizable.

Extrapair paternity.—We scored DNA fingerprints from 137 chicks and 17 embryos sampled

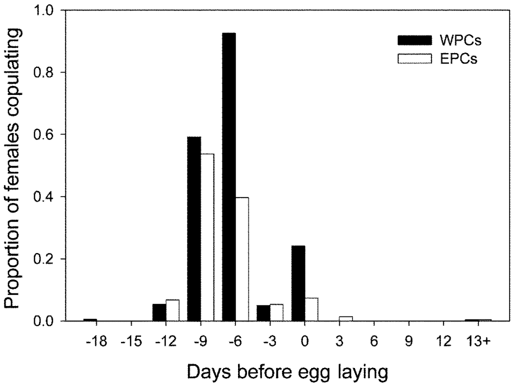

Fig. 2. Temporal overlap of within-pair copulations (WPCs) and extrapair copulations (EPCs) for female Waved Albatrosses shown as the proportion of breeding females that had at least one copulation in each three-day interval.

during the three breeding seasons. Of the 154 sampled offspring, 128 had zero or one band that could not be attributed to either social parent; the rest ($n = 26$) had two to six unattributable bands. Band-sharing among first-order relatives (mother–offspring) ranged from 0.513 to 0.944, whereas band-sharing among unrelated birds ranged from 0.067 to 0.471 (Fig. 3). Band-sharing among social father–offspring dyads fell into two discrete groups. One group ($n = 128$) resembled first-order relatives closely, with values ranging from 0.452 to 0.867; band-sharing values for the second group all fell below 0.424 and were similar to band-sharing among unrelated individuals (range: 0.158–0.380). All 26 offspring in this second group were those that had two or more novel bands. On the basis of low band-sharing values and the presence of two or more novel bands, we concluded that all 26 social fathers of these chicks were not the genetic fathers of their putative offspring (Fig. 4).

Putative mothers paired with offspring that had two or more unattributable bands all had band-sharing values between 0.538 and 0.848, in keeping with values for known first-order relatives (Fig. 4). We conclude that these social mothers were in all cases the genetic mothers of the offspring they attended. Overall, our estimate for the frequency of extrapair paternity in Waved Albatrosses was 16.9% (26 of 154). The 95% binomial confidence interval for this estimate is 0.113–0.238. Extrapair fertilization frequency did not differ between years (n_{2000} = 7 of 33 [21.2%], n_{2001} = 10 of 68 [14.7%], n_{2002} = 9 of 54 [16.7%]; G-test: $G = 0.66$, df = 2, $P = 0.719$), nor did EPF rate differ for hatchlings versus embryos (n_h = 24 of 137 [17.5%], n_e = 2 of 17 [11.8%]; G-test: $G = 0.38$, df = 1, $P = 0.538$).

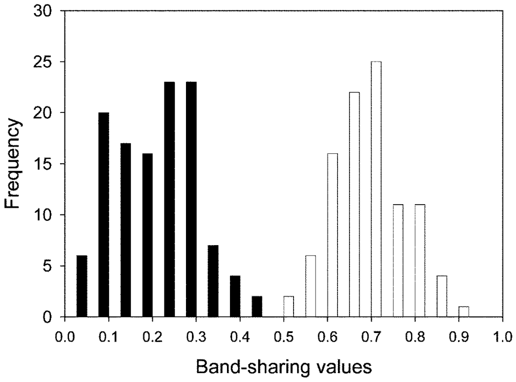

FIG. 3. Band-sharing values between dyads of Waved Albatrosses. Filled bars depict band-sharing for dyads of chicks with unrelated adults. Open bars show band-sharing between mothers and chicks.

Probability of misassigning an unrelated male as father was 0.001, and probability of misassigning an uncle as father was 0.074. Average number of novel bands per unexcluded offspring was 0.039, and we scored an average of 15.1 bands per lane, yielding an estimated mutation rate of 0.039/15.1, or 0.003 mutations per locus per meiotic event. This value is similar to those in other seabirds (e.g. 0.008; Mauck et al. 1995) and is close to that estimated in Waved Albatrosses previously (0.004; Huyvaert et al. 2000). We conclude that mutation can account for the single novel band of five unexcluded offspring.

Fitness consequences of protandry.—Males were assigned as genetic fathers in 18 of the 26 (69.2%) cases of extrapair paternity. We were able to attribute all ($n = 17$) or all but one ($n = 1$) offspring band to either the mother or assigned father and all chick–genetic father band-sharing values (mean = 0.666 ± 0.079, range: 0.519–0.824) were greater than the cutoff value, 0.424. Of

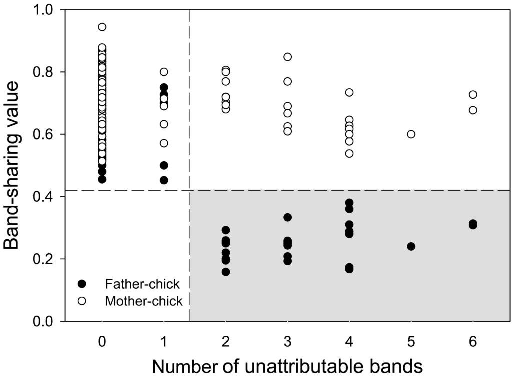

FIG. 4. Relationship between band-sharing and number of unattributable bands within Waved Albatross social families. The dashed lines and shaded box highlight the criteria used to determine extrapair parentage. Extrapair paternity was indicated in cases with social father–chick dyads with band-sharing values falling below 0.424 (horizontal line) and with two or more unattributable bands (vertical line). All cases of extrapair paternity fall within the shaded box.

the 18 males assigned as extrapair fathers, 17 attempted to breed with their social mate, 9 of these attempts led to hatched chicks, and 2 were cuckolded in the same year that they cuckolded another bird.

A male's extrapair paternity did not depend on the relative timing of his arrival (logistic regression: $\chi^2_{2000} = 0.76$, $P = 0.38$; $\chi^2_{2001} = 0.09$, $P = 0.77$; $\chi^2_{2002} = 2.3$, $P = 0.13$), though the number of known sires in each analysis was small ($n_{2000} = 4_{\text{epf}}$, $23_{\text{no epf}}$; $n_{2001} = 6, 46$; $n_{2002} = 4, 36$). However, using a proxy measure for fertilization date, EPF events themselves occur earlier than WPFs (Kolmogorov-Smirnov test: $P < 0.10$; $\text{mean}_{\text{epf}} = 5.26 \pm 9.86$, median = 1 day, range: 39 days before to 19 days after, $n_{\text{epf}} = 23$; $\text{mean}_{\text{wpf}} = 0.03 \pm 7.84$, median = 8 days, range, 10 days before to 21 days after, $n_{\text{wpf}} = 96$).

Hatchling and near-fledging matches made using fingerprinting indicate that reproductive success did not differ between extrapair and other males across all years combined (chi-square test: $\chi^2 = 0.360$, df = 1, $P = 0.546$). This measure of reproductive success did not differ for any single year (Fisher's exact test: $P > 0.60$ in all cases), either, though consistently fewer fledglings were produced per known extrapair male than by other males (2000: 0.250 young per extrapair sire vs. 0.581 young; 2001: 0.500 vs. 0.554; 2002: 0.167 vs. 0.186). Overall male reproductive success, measured as a male having sired any offspring that lived to fledge, was not related to either a male's arrival in relation to that of his social mate or his Julian arrival date (chi-square test: date in relation to social mate: $\chi^2_{2000} = 0.19$, $P = 0.67$; $\chi^2_{2001} = 1.72$, $P = 0.19$; $\chi^2_{2002} = 2.8$, $P = 0.09$; Julian date: $\chi^2_{2000} = 1.24$, $P = 0.27$; $\chi^2_{2001} = 1.4$, $P = 0.24$; $\chi^2_{2002} = 2.7$, $P = 0.10$).

Discussion

Protandry is pronounced in the Waved Albatross. In general, male breeders arrive earlier than female breeders, and males that retain social mates return to the breeding colony 6–10 days before the female does. Earlier arrival at breeding sites by male Waved Albatrosses is similar to the breeding biology of other migratory species, including many passerines (e.g. Mills 2005) and a nonpasserine (White Stork [*Ciconia ciconia*]; Creutz 1985, as cited in Tryjanowski et al. 2004). Several hypotheses have been proposed to explain protandry in birds. Among them, the mate opportunity hypothesis proposes that earlier arrival affords males more copulation opportunities that might lead to additional reproductive success via EPFs. Extrapair behavior is not expected in socially monogamous taxa with high adult survival and essential male care, because males are believed to respond to uncertain paternity in the current brood through abandonment when their expectation of future reproduction is high (Mauck et al. 1999), yet we found that >60% of observed copulations by breeding female Waved Albatrosses were EPCs and >70% of copulations by breeding males were EPCs. In both sexes, arrival timing was negatively correlated with EPC frequency. As we predicted, males arriving many days earlier than their social mates participated in more EPCs than males arriving relatively later, though male Julian arrival date was not correlated with EPC number. This suggests that one advantage to males of arriving early is not having a social mate to guard or to be guarded by, which frees the male for EPCs.

Alternatively, a male may arrive earlier than his mate to ensure that he is available for WPCs upon her arrival or so that he may take up mate-guarding as soon as possible, thus reducing the time available to the female to pursue her own EPCs. Reunions of social mates in the colony can be immediate if the male is present when the female arrives, or can take several hours to days if the male is not at the appropriate site when the female returns (K. Huyvaert et al. pers. obs.). Also consistent with male mate-guarding is the finding that female Waved Albatrosses that arrived many days after their social mates had fewer EPCs than females that arrived before or a few days after their mates. Although divorce rates are low in this species (2 of 254 [0.008] pairings ended in divorce), the probability of mate retention is positively associated with the amount of time mates share in the colony before egg laying (K. Huyvaert et al. unpubl. data). Thus, early arrival by males may be a tactic that retains a social mate, assures paternity, or increases copulation opportunities—any of which could enhance reproductive success.

Although the incidence of EPC is widespread among birds (for overviews, see Griffith et al. 2002, Westneat and Stewart 2003), frequency of EPCs among all copulations observed here was high, compared with those of other shorebirds and seabirds. For example, only 21% of observed copulations were EPCs in Red-billed Gulls (*Larus novaehollandiae scopulinus*; Mills 1994), 15% were EPCs in Spotted Sandpipers (*Actitis macularius*; Colwell and Oring 1989), 2.4% were EPCs in Northern Fulmars (which are closely related to albatrosses; Hunter et al. 1992), and no EPCs were observed in King Penguins (*Aptenodytes patagonicus*; Olsson et al. 2001). Some aspects of these species' breeding biology clearly differ from that of Waved Albatrosses. King Penguins, for example, have high divorce rates resulting from high costs of mate retention that are linked to the harsh breeding environment and associated time constraints on breeding (Bried et al. 1999). At the same time, effective mate-guarding by male King Penguins, associated with a lack of nest site or territory, is believed to explain the absence of EPCs (Olsson et al. 2001).

By contrast, the Waved Albatross may not be subject to similar time constraints, given its tropical breeding habitat, and the incidence of territoriality could help explain this species' relatively high EPC rate in light of the correlations between EPCs and arrival timing. Waved Albatrosses are territorial in the sense that they return to sites within a few meters of the previous year's nesting site, but territoriality is necessarily fluid because some birds must travel hundreds of meters through other territories to find suitable wind-aided take-off sites. The material benefit of arriving early may include acquisition of territories with easy access to wind-aided take-off sites. Extrapair copulations might then be an epiphenomenon of territoriality, as Harris (1973) originally proposed: early-arriving birds get the best sites, the best sites must be aggressively defended, and territory defense may include apparent EPCs; thus, early-arriving males have relatively more EPCs. However, this explanation can be supported only when EPCs are negatively associated with early arrival in comparison with other males; we found this to be the case in 2002, but not in 2000 or 2001 (see Table 2). This lack of strong support for a material benefit to early arrival motivates a closer look at possible direct fitness advantages, such as enhanced reproductive success via EPFs.

Using our behavioral "completion" criterion, 36–42% of EPCs by breeding female Waved Albatrosses were classified as successful. Also, EPCs and WPCs in Waved Albatrosses overlap temporally, which suggests that EPCs occur when females are fertilizable; both findings prompt examining the fitness consequences of EPC behavior. Most explanations advanced for intraspecific variation in EPCs and EPFs among males deal with variability in age, experience, body size, or other traditional measures of quality (see Westneat and Stewart [2003] for summary). The mate opportunity hypothesis measures male quality as relative arrival time, with earlier arrival enhancing reproductive success if extra mating opportunities lead to EPFs. Because EPFs cannot always be predicted reliably from observed EPC behavior (Dunn and Lifjeld 1994; but see Birkhead and Møller 1992, 1995), molecular genetic determinations of paternity should better address critical fitness-related predictions of hypotheses. Using multilocus minisatellite DNA fingerprinting, 16.9% (26 of 154; 14–21% across years) of Waved Albatross offspring sampled in our study area were EPY. Extrapair paternity is generally absent in procellariiforms, but the present study and an earlier one of smaller scope (Huyvaert et al. 2000) suggest that this species is different. Given that Waved Albatrosses meet the essential requirements for a protandry-mediated fitness advantage of EPCs, EPCs are correlated with early arrival, EPCs are timed to lead to fertilizations, and EPFs occur regularly, we asked: Is timing of arrival linked to extrapair paternity and does extrapair paternity confer a direct fitness advantage to males through enhanced reproductive success?

Early arrival is variably associated with components of male fitness in other bird species. In American Redstarts (*Setophaga ruticilla*), for example, breeding males arrived earlier than those that did not breed, but arrival timing was not different between males that fledged chicks and those that failed (Lozano et al. 1996). On the other hand, early arrival appeared to enhance reproductive success for male Sedge Warblers (*Acrocephalus schoenbaenus*), given that males siring EPY arrived earlier than those that did not and the number of females with whom a male mated tended to be larger for males siring EPY (Langefors et al. 1998), though no direct comparison of fledging rate was made between cuckolding males and other males. As with American Redstarts, we did not find support for a link between early arrival and reproductive success: we found no difference in arrival timing between extrapair sires and other males, nor did we find a difference in the average number of chicks fledged per male between the groups. Further, no connection exists between arrival timing and overall reproductive success, which eliminates improved within-pair reproduction as an explanation for protandry in Waved Albatrosses.

The laying dates of Waved Albatross eggs that were extrapair offspring tended to be earlier than those of all other eggs, which hints at a fitness advantage of early arrival. Small sample sizes or unpredictable environmental conditions among years may explain the lack of a relationship between arrival, extrapair (as well as within-pair) paternity, and reproductive success. Further, El Niño–Southern Oscillation conditions that prevailed during chick rearing in the 2002 breeding season (Climate Prediction Center; see Acknowledgments) drastically reduced numbers of fledglings (and our testable sample size in that year), likely because of starvation. If losses to mortality were random with respect to extrapair paternity, then a final possibility explaining our results is that EPFs involve a trade-off for extrapair sires: if EPF probability is positively correlated with paternity losses at home, then EPFs will not contribute significantly to overall variance in male reproductive success, such that we should not find important differences between extrapair sires and other types of males (e.g. Ketterson et al. 1998), as is the case in the present study. On the other hand, mortality may not be random with respect to extrapair paternity when environmental factors cause late-season mortality. Extrapair copulations tend to occur earlier, so EPY may have more time to fledge before poor conditions develop than later-hatching, within-pair young. We have little data to assess how often climate factors cause late-season mortality, though seasonal variation in arrival timing exists and may be attributable to differences in sea-surface temperatures at the beginning of the breeding season. Mean monthly sea-surface temperatures for February and March (Climate Prediction Center; see Acknowledgments), just before Waved Albatrosses return, appear to be negatively associated with the return date of the first banded birds, though small sample sizes limit inferences. In 2000, the first banded male returned on 31 March, and the February–March mean temperature was 25.95°C. These figures were 31 March and 26.68°C for 2001 and 12 April and 26.80°C for 2002.

Taken together, our data provide evidence of an advantage of early arrival at the breeding colony in terms of added EPC opportunities, but only hint at a direct fitness advantage of early arrival for male Waved Albatrosses. Although EPFs occur regularly in our study population, they cannot be attributed to protandrous arrival at the breeding colony, and the selective advantages of EPF as an alternative reproductive strategy may be more closely tied to male territoriality or to benefits accrued by females. Finally, the benefits of protandry are more likely associated with mate guarding and paternity assurance, benefits accrued "at home," rather than benefits to reproductive success gained through EPFs somewhere else.

Acknowledgments

We are grateful to our field assistants for their careful work observing copulation behavior. We appreciate insightful comments from D. B. Lank, G. Miller, and an anonymous reviewer. Funding for this study came from the American Museum of Natural History Chapman Fund, the Des Lee Collaborative Vision in Zoological Studies and the International Center for Tropical Ecology at the University of Missouri-St. Louis (UMSL), the Field Research for Conservation Program at the St. Louis Zoological Park, the National Science Foundation through grants DEB 98-06606 and 0235818 to D.J.A., and Wake Forest University. Additional fellowship support to K.P.H. during the study came from The Ohio State University (OSU) and UMSL. This study was conducted with permission to handle and sample birds from the Galápagos National Park Service and with logistical support from the Charles Darwin Research Station, particularly from P. Robayo, and TAME airlines. All observation, capture, and sampling procedures were approved by the Institutional Animal Care and Use Committees at OSU and UMSL. We used weather data from the following U.S. National Weather Service Climate Prediction Center websites: (2004) www.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ensoyears.html, and (2005) www.cpc.ncep.noaa.gov/data/indices/sstoi.indices.

Literature Cited

Altmann, J. 1974. Observational study of behaviour: Sampling methods. Behaviour 49:227–267.

Anderson, D. J., K. P. Huyvaert, V. Apanius, H. Townsend, C. L. Gillikin, L. D. Hill, F. Juola, E. T. Porter, D. R. Wood, C. Lougheed, and H. Vargas. 2002. Population size and trends of the Waved Albatross *Phoebastria irrorata*. Marine Ornithology 30: 63–69.

Austin, J. J., and D. T. Parkin. 1996. Low frequency of extra-pair paternity in two colonies of the socially monogamous Short-tailed Shearwater *Puffinus tenuirostris*. Molecular Ecology 5:145–150.

Awkerman, J. A., K. P. Huyvaert, and D. J. Anderson. 2005. Mobile incubation in Waved Albatrosses (*Phoebastria irrorata*): Associated hatching failure and artificial mitigation. Avian Conservation and Ecology 1(1). [Online.] Available at www.ace-eco.org/vol1/iss1/art2/.

Birkhead, T. R., and A. P. Møller. 1992. Sperm Competition in Birds: Evolutionary Causes and Consequences. Academic Press, San Diego, California.

Birkhead, T. R., and A. P. Møller. 1995. Extra-pair copulation and extra-pair paternity in birds. Animal Behaviour 49:843–848.

Bried, J., F. Jiguet, and P. Jouventin. 1999. Why do *Aptenodytes* penguins have high divorce rates? Auk 116:504–512.

Charnov, E. L. 1982. The Theory of Sex Allocation. Princeton University Press, Princeton, New Jersey.

Colwell, M. A., and L. W. Oring. 1989. Extra-pair mating in the Spotted Sandpiper: A female mate acquisition tactic. Animal Behaviour 38:675–684.

Creutz, G. 1985. Der Weißstorch *Ciconia ciconia*. A. Ziemsen Verlag, Wittenberg Lutherstadt.

Dearborn, D. C., A. D. Anders, and P. G. Parker. 2001. Sexual dimorphism, extra-pair fertilizations, and operational sex ratio in Great Frigatebirds (*Fregata minor*). Behavioral Ecology 12:746–752.

Dunn, P. O., and A. Cockburn. 1998. Costs and benefits of extra-group paternity in Superb Fairy-Wrens. Pages 147–161 *in* Avian Reproductive Tactics: Female and Male Perspectives (P. G. Parker and N. T. Burley, Eds.). Ornithological Monographs, no. 49.

Dunn, P. O., and J. T. Lifjeld. 1994. Can extra-pair copulations be used to predict extra-pair paternity in birds? Animal Behaviour 47:983–985.

Emlen, S. T., and L. W. Oring. 1977. Ecology, sexual selection and the evolution of mating systems. Science 197:215–223.

Fridolfsson, A.-K., and H. Ellegren. 1999. A simple and universal method for molecular sexing of non-ratite birds. Journal of Avian Biology 30:116–121.

Griffith, S. C., I. P. F. Owens, and K. A. Thuman. 2002. Extra pair paternity in birds: A review of interspecific variation and adaptive function. Molecular Ecology 11:2195–2212.

Harris, M. P. 1973. The biology of the Waved Albatross *Diomedea irrorata* of Hood Island, Galapagos. Ibis 115:483–510.

Hunter, F. M., T. Burke, and S. E. Watts. 1992. Frequent copulation as a method of paternity assurance in the Northern Fulmar. Animal Behaviour 44:149–156.

Huyvaert, K. P. 2004. Social and genetic dynamics of extra-pair mating in Waved Albatrosses (*Phoebastria irrorata*). Ph.D. dissertation, University of Missouri, St. Louis.

Huyvaert, K. P., D. J. Anderson, T. C. Jones, W. Duan, and P. G. Parker. 2000. Extra-pair paternity in Waved Albatrosses. Molecular Ecology 9:1415–1419.

Jeffreys, A. J., V. Wilson, and S. L. Thein. 1985a. Hypervariable 'minisatellite' regions in human DNA. Nature 314:67–73.

Jeffreys, A. J., V. Wilson, and S. L. Thein. 1985b. Individual-specific 'fingerprints' of human DNA. Nature 316:76–79.

Ketterson, E. D., and V. Nolan, Jr. 1976. Geographic variation and its climatic correlates in the sex ratio of eastern-wintering Dark-eyed Juncos (*Junco hyemalis hyemalis*). Ecology 57:679–693.

Ketterson, E. D., P. G. Parker, S. A. Raouf, V. Nolan, Jr., C. Ziegenfus, and C. R. Chandler. 1998. The relative impact of extra-pair fertilizations on variation in male and female reproductive success in Dark-eyed Juncos (*Junco hyemalis*). Pages 81–101 *in* Avian Reproductive Tactics: Female and Male Perspectives. (P. G. Parker and N. T. Burley, Eds.). Ornithological Monographs, no. 49.

Langefors, A., D. Hasselquist, and T. von Schantz. 1998. Extra-pair fertilizations in the Sedge Warbler. Journal of Avian Biology 29:134–144.

Longmire, J. L., A. K. Lewis, N. C. Brown, J. M. Buckingham, L. M. Clark, M. D. Jones, L. J. Meincke, J. Meyne, R. L. Ratliff, F. A. Ray, and others. 1988. Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family Falconidae. Genomics 2:14–24.

Lozano, G. A., S. Perreault, and R. E. Lemon. 1996. Age, arrival date and reproductive success of male American Redstarts *Setophaga ruticilla*. Journal of Avian Biology 27:164–170.

Lynch, M. 1990. The similarity index and DNA fingerprinting. Molecular Biology and Evolution 7:478–484.

Martin, P., and P. Bateson. 1993. Measuring Behaviour: An Introductory Guide, 2nd ed. Cambridge University Press, Cambridge, United Kingdom.

Mauck, R. A., E. A. Marschall and P. G. Parker. 1999. Adult survival and imperfect assessment of parentage: Effects on male parenting decisions. American Naturalist 154:99–109.

Mauck, R. A., T. A. Waite, and P. G. Parker. 1995. Monogamy in Leach's Storm Petrel: DNA-fingerprinting evidence. Auk 112: 473–482.

Mills, A. M. 2005. Protogyny in autumn migration: Do male birds "play chicken"? Auk 122:71–81.

Mills, J. A. 1994. Extra-pair copulations in the Red-billed Gull: Females with high-quality, attentive males resist. Behaviour 128:41–64.

Morbey, Y. E., and R. C. Ydenberg. 2001. Protandrous arrival timing to breeding areas: A review. Ecology Letters 4:663–673.

Olsson, O., J. Bonnedahl, and P. Anker-Nilssen. 2001. Mate switching and copulation behaviour in King Penguins. Journal of Avian Biology 32:139–145.

Piper, W. H., and P. P. Rabenold. 1992. Use of fragment-sharing estimates from DNA fingerprinting to determine relatedness in a tropical wren. Molecular Ecology 1:69–78.

Rabenold, P. P., K. N. Rabenold, W. H. Piper, M. D. Decker, and J. Haydock. 1991. Using DNA fingerprinting to assess kinship and genetic structure in avian populations. Pages 611–620 *in* The Unity of Evolutionary Biology: Proceedings of the IV International Congress on Systematics and Evolutionary Biology (E. C. Dudley, Ed.). Dioscorides Press, Portland, Oregon.

Rabenold, P. P., K. N. Rabenold, W. H. Piper, J. Haydock, and S. W. Zack. 1990. Shared paternity revealed by genetic analysis in cooperatively breeding tropical wrens. Nature 348:538–540.

Reynolds, J. D., M. A. Colwell, and F. Cooke. 1986. Sexual selection and spring arrival times of Red-necked and Wilson's phalaropes. Behavioral Ecology and Sociobiology 18:303–310.

Schwartz, M. K., D. J. Boness, C. M. Schaeff, P. Majluff, E. A. Perry, and R. C. Fleischer. 1999. Female-solicited extra-pair matings in Humboldt Penguins fail to produce extra-pair fertilizations. Behavioral Ecology 10: 242–250.

Stutchbury, B. J. M. 1998. Breeding synchrony best explains variation in extra-pair mating system among avian species. Behavioral Ecology and Sociobiology 43:221–222.

Tickell, W. L. N. 1968. Movements of Wandering Albatrosses *Diomedea exulans*. Emu 68:1–20.

Tryjanowski, P., T. H. Sparks, J. Ptaszyk, and J. Kosicki. 2004. Do White Storks *Ciconia ciconia* always profit from an early return to their breeding grounds? Bird Study 51: 222–227.

Westneat, D. F., and I. R. K. Stewart. 2003. Extra-pair paternity in birds: Causes, correlates, and conflict. Annual Review of Ecology and Systematics 34:365–396.

Wetton, J. H., R. E. Carter, D. T. Parkin, and D. Walters. 1987. Demographic study of a wild House Sparrow population by DNA fingerprinting. Nature 327:147–149.

Zar, J. H. 1999. Biostatistical Analysis, 4th ed. Prentice Hall, New Jersey.

Associate Editor: D. B. Lank