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ORIGINAL ARTICLE

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Mate guarding, copulation strategies and paternity in the sex-role reversed, socially polyandrous red-necked phalarope *Phalaropus lobatus*

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Abstract In a recent review, Westneat and Stewart (2003) compiled evidence that extra-pair paternity results from a three-player interaction in which sexual conflict is a potent force. Sequentially polyandrous species of birds appear to fit this idea well. Earlier breeding males may attempt to use sperm storage by females to obtain paternity in their mate's subsequent clutches. Later-breeding males may consequently attempt to avoid sperm competition by preferring to pair with previously unmated females. Females may bias events one way or the other. We examined the applicability of these hypotheses by studying mating behavior and paternity in red-necked phalaropes (*Phalaropus lobatus*), a sex-role reversed, socially polyandrous shorebird. Male red-necked phalaropes guarded mates more strongly than other shorebirds. Males increased within-pair copulation attempts during their mate's fertile period, and maintained or further increased attempts towards the end of laying, suggesting an attempt to fertilize the female's next clutch; these attempts were usually thwarted by the female. Paired males sought extra-pair copulations with females about to re-enter the breeding pool. Multilocus DNA fingerprinting showed that 6% of clutches (4/63) each contained one chick sired by a male other than the incubator, producing a population rate of these events of 1.7% (n=226 chicks). Male mates had full paternity in all first clutches (n=25) and 15 of 16 monogamous replacement clutches. In contrast, 3 of 6 clutches of second males contained extra-pair young likely fathered by the female's previous mate. Previously mated female phalaropes may employ counter-strategies that prevent later mating males from discriminating against them. The stability of this polyandrous system, in which males provide all parental care, ultimately may depend on females providing males with eggs containing primarily genes of the incubating male, and not a previous mate.

Keywords DNA fingerprinting · Paternity · *Phalaropus* · Polyandry · Sex role reversal

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Introduction

Patterns of paternity in birds have received considerable attention recently because they dramatically change our understanding of mating behavior in this taxon. Furthermore, the wide variation in the frequency of extra-pair fertilizations (EPF), ranging from 0 to 85% of offspring, offers an opportunity to test general ideas for the ecology of mating behavior. In a recent review, Westneat and Stewart (2003) compiled evidence supporting the idea that extra-pair paternity arises from a three-player interaction. This interaction is best understood in terms of sexual conflict theory (Parker 1970; Gowaty 1996; Westneat 2000), in which the reproductive strategy of each participant may be at odds with one or more of the others. A female benefits from producing eggs with a high likelihood of hatching and resulting in young with high fitness. Each male benefits as well from fertilizing as many eggs as possible. Sexual conflict arises when the male or males attempting to fertilize a female's eggs do not have all the characteristics beneficial to her or her young (Birkhead and Møller 1996; Møller 2000; Griffith et al. 2002; Westneat and Stewart 2003).

Predicting the outcome of this interaction is complicated by overlaying social opportunities, ecological limitations, and the relative "leverage" of each sex at particular reproductive stages (Brown et al. 1997). For example, territorial behavior may limit mating opportunities for females if females obtain most of the resources they need on the social mate's territory (Birkhead and Møller 1992), but in situations where they leave the territory, extra-pair paternity (EPP) may be quite high (Kempenaers et al. 1992; Smiseth and Amundsen 1995). Mate-guarding can reduce EPP risk (Møller and Birkhead 1993; Westneat 1994; Komdeur et al. 1999), but females may escape their mate's guarding (Johnsen et al. 1998), especially in dense cover (Davies 1992; Mays 2001). Males can increase paternity assurance by increased copulation rates (Birkhead and Møller 1992), but strategic timing of an extra-pair copulation (EPC) can be effective because one EPC can have a higher sperm count than a within-pair copulation (Michl et al. 2002; Pizzari et al. 2003). Social polygyny can affect the interactions between the sexes in some situations, leading to increased EPP (e.g., Dunn and Robertson 1993; Westneat 1993).

Paternity is especially interesting in sex-role reversed species with precocial young, where a male provides all parental care for the eggs and young. In some birds, males apparently provision gauged by their level of paternity, perhaps because other individuals can compensate for the reduction in care (Burke et al. 1989; Dixon et al. 1994; Dunn and Cockburn 1996). In most polyandrous shorebirds, however, only the male provides care after egg-laying. Reduction of care may be constrained in such systems, and thus males should invest heavily in paternity assurance behaviors (Møller 2000; Griffith et al. 2002). This social mating system also provides multiple mating opportunities for the female, and, as a result, sperm competition could affect male fitness. The current model of sperm competition in birds includes sperm storage by females (Birkhead and Møller 1992), mixing of sperm from multiple sources (Colegrave et al. 1995), passive sperm loss over time (Birkhead and Fletcher 1995), high-mobility sperm (Birkhead et al. 1999), and sperm allocation strategies by males (Pizzari et al. 2003). Co-mates or second mates of polyandrous females thus have both EPP risks and opportunities. Their evolved behaviors should minimize extra-pair young (EPY) in their care and promote EPY in the care of others, a mixed reproductive strategy (Trivers 1972).

EPP occurs at low frequencies in socially polyandrous species of shorebirds with uni-parental male care (5–7% of all chicks: Oring et al. 1992; Owens et al. 1995; Emlen et al. 1998; Dale et al. 1999). Two studies of territorial species identified the sires of these young. In the sequentially polyandrous spotted sandpiper (*Actitis macularia*), EPP was obtained by a female's previous mates via stored sperm following rapid mate-switching (short time between a female's successive clutches) (Oring et al. 1992; see also Mee et al. 2004). In the simultaneously polyandrous wattled jacana (*Jacana jacana*), "co-mates" were the source of EPP, through ongoing copulations with non-incubating males in the female's harem (Emlen et al. 1998). In both species, females control access to mates by monopolizing access to resources (Emlen and Oring 1977), and males establish sub-territories within the territories of the larger, more aggressive females. Neither spotted sandpiper nor jacana males guard mates directly (Oring et al. 1994), because of female dominance to males and restricted movements of males on a female's territory. Instead, males reduce EPP risk through strategic timing of copulation to coincide with the female's fertile period (Oring et al. 1994) and/or elevated copulation rates (Emlen et al. 1998).

By contrast, three phalarope species and the dotterel (*Charardrius morinellus*) are sequentially polyandrous with obligatory male parental care, but non-territorial (Hildén and Vuolanto 1972; Schamel and Tracy 1977; Kålås and Byrkjedal 1984; Colwell 1986). Factors promoting the potential for EPP in phalaropes include (see Colwell and Jehl 1994; Rubega et al. 2000; Tracy et al. 2002): (1) lack of territorial defense, although both sexes are aggressive to intruding conspecifics that approach the pair; typically, males repulse intruding females and vice-versa; (2) clumped feeding and nesting, making them semi-colonial; (3) a short time between successive clutches (4–8 days); (4) male solo nest-building activities during the laying period, which reduces potential mate-guarding; (5) a compressed nesting season in arctic-nesting species, making nesting more synchronous; and (6) substantially larger testes and longer sperm tails than similar-sized monogamous shorebirds (Briskie et al. 1997; Johnson and Briskie 1999). Factors inhibiting the potential for EPP in phalaropes include: (1) use of open habitats where it is relatively easy to track the mate location and activity; (2) close association of pair members during pre-laying and laying; and (3) apparent choosiness of males in avoiding pairing with previously-mated females (Whitfield 1990; Schamel et al. 2004).

To date, two studies have examined paternity in phalaropes and one in dotterels. No evidence of EPP was found in 17 broods of Wilson's phalaropes (*Phalaropus tricolor;* Delehanty et al. 1998). EPP was found in 6 of 18 broods of red phalaropes (*P. fulicarius*), including 50% of clutches (*n*=12) produced later in the season (Dale et al. 1999). In the dotterel, 2 EPP chicks were found among 22 broods, both from later clutches (Owens et al 1995). A higher rate of EPP in later clutches suggests sperm storage from previous males. However, sires of EPP chicks were not identified in these studies, and EPCs associated with re-pairing or during later pair bonds may have accounted for these chicks.

We studied mating behavior and paternity in the red-necked phalarope (*Phalaropus lobatus*). Based on the characteristics of the species and the three studies of paternity in similar species, we predicted that: (1) the overall EPP rate should be low, since high levels of paternity assurance should be required for male-only parental care (Ketterson and Nolan 1994; Møller 2000); (2) second clutches should have higher rates of EPP than first clutches, due to stored sperm and rapid mate-switching (Oring et al. 1992); (3) because this is a non-territorial species, paired males should mate guard to prevent copulations from competitors (Birkhead and Møller 1992); (4) males should protect their paternity with elevated copulation attempts and rates (Birkhead and Møller 1992), because the increasing time they spend in nest platform construction precludes continuous mate guarding; second males might be particularly like to use this tactic, to prevent fertilizations from stored sperm; (5) infertility rates should be lower in second clutches, in which increased sperm competition is expected; and (6) males should pursue a mixed reproductive strategy (Trivers 1972) through increased within-pair copulations with their females just prior to clutch completion and through copulations with females seeking second mates (Fitch and Shugart 1984).

Methods

Field methods

Fieldwork was carried out at Cape Espenberg (66°30′N, 163°30′W), on the northern Seward Peninsula, in western Alaska, during late May through mid-July 1977–1979 and 1994–1999. The habitat consisted of wet coastal tundra, interspersed with small ponds and wet sedge marsh, where phalaropes nest and rear young during the short arctic summer (Schamel and Tracy 1987). We searched a 2-km^2 area for red-necked phalarope pairs and nests, attempting to find nests early in laying by following pre-incubating pairs daily. Adults were captured with hoop nets, nest traps (Schamel and Tracy 1977), or long-handled dip nets strung with mist netting (Reynolds 1987), and received a unique combination of colored bands. We recorded nest and pair bond histories of marked phalaropes.

We made daily focal observations to determine potential mate guarding behavior, and within- and extra-pair copulation rates for 36 pairs (188.7 h) in 1977–1979 and 63 pairs (149.0 h) in 1996–1999. At 1-min intervals, we estimated the distance between pair members, to the nearest 1 m, and recorded at that time whether a pair member had approached or moved away from its partner since the last scan. Since birds can move parallel to one another, the summed proportion of these movements does not equal 1.0. Copulations were initiated by both males and females (Rubega et al. 2000), and were considered successful if cloacal contact occurred, which required 8–12 s. Females refused copulations by standing abruptly, spinning in circles, or walking away quickly.

We established the laying stage for each pair by direct observation of egg laying dates, or by backdating eggs by their floatation characteristics in a clear container of water (Sandercock 1998). We scaled the first day of laying as day 0. On average, one egg is laid per day (Rubega et al. 2000), thus fertilization was most likely on days −1, 0, 1, and 2 for a typical clutch of 4. We eliminated day 1 of the pair bond from analyses because: (1) behavior patterns were qualitatively different early in pairing, with females typically following males at distances <10 cm; and (2) there was a variable amount of time from pairing to egg-laying.

Intense predation on eggs in 1994–1996 resulted in 90–95% loss of clutches, and thus paternity data from only a few chicks. In response, we deployed predator exclosures (Mabee and Estelle 2000) in 1997–1999, and collected eggs after 5–9 days of incubation. We visually inspected collected eggs for embryos, and recorded all eggs with no embryo development as infertile. Birkhead et al. (1993) suspected that early mortality of embryos in house sparrows (*Passer domesticus*) resulted from egg neglect at the start of incubation. In contrast, eggs of arctic-nesting shorebirds remain viable without incubation for 2–3 days, even with temperatures below freezing (Mehlum 1991). We suspect that completely undeveloped eggs in our study were, therefore, probably infertile. Blood samples from all adults (30–100 μl) and four chicks (10–20 μl) were stored in lysis buffer, and tissue samples from 228 embryos were stored in brine solution (Seutin et al. 1991). DNA analyses of paternity were based upon 45 clutches with blood samples from both putative parents and 18 clutches with blood samples from males only. This sample consisted of 25 "first" clutches, 16 same-pair "replacement" clutches following clutch loss, 6 clutches laid by polyandrous females with second mates ("polyandrous" clutches), and 16 clutches of unknown status.

Paternity analysis

We extracted DNA using a salt extraction procedure modified from Miller et al. (1988), and cleansed excess protein from DNA using phenol/chloroform. DNA was digested with *Hae*III (Westneat 1990), separated on agarose gels, transferred to nylon membranes, and probed using radiolabeled Jeffreys 33.15 and M13 minisatellite probes, following standard protocols (Westneat 1990, 1993) to produce DNA fingerprints. Bands were scored as in Westneat (1990). Chicks were compared with each putative parent and, in the case of polyandrous clutches, with the female's first mate. A band not occurring in the DNA profiles of either putative parent was defined as "novel". Fingerprints for each probe were scored separately, then combined for final band-sharing analysis. We determined a band-sharing coefficient (D) between individuals by $D=2s/(a+b)$ (Wetton et al. 1987), where s is the number of bands the two profiles have in common, and a and b are the total number of bands in each individual, respectively. We established the confidence limits (CL) for novel bands by solving for m in the Poisson distribution (Westneat 1990) by averaging across 0–8 novel bands (m=0.988). This figure was used to set the upper 99% CL of this distribution at 3 novel bands. When DNA was only available from the male parent, we followed the scoring protocols of Dale et al. (1999), and did not define novel bands for these nests.

Statistical analyses

We summarized behavioral data for each pair per day as one rate (copulations and copulation attempts) or proportion (pair associations and movements). We calculated copulation rates for pairs with 5 or more minutes of observations per day. Because phalaropes are not territorial, and thus not tied to a specific site, we could not collect complete sets of daily observations on the same pair from pre-laying through clutch completion. Our analyses of behavioral rates with respect to laying times used repeated measures with PROC MIXED (SAS 1999) with the Satterthwaite approximation for denominator degrees of freedom. We treated laying stage as a fixed effect; each pair was treated as a separate subject; data were pooled within pairs (n=16). In four cases, behavioral data came from birds that formed pairs in successive years; we treated these as separate pairs. One- and two-way repeated measures ANOVAs or ANCOVAs and t-tests, as appropriate, were used to examine differences in distance between pair members, rates of approach categories, and copulation rates with respect to time of the laying cycle and clutch type. Bonferroni adjusted tests or contrasts were used to locate sources of variation. Means are given with standard errors, except where noted. Statistics were calculated using SAS (SAS 1999). Because our data on copulation rates were skewed, these data were log-transformed and 0.01 was added to each value prior to statistical analyses. Fisher's Exact Test was used when expected values in a contingency table invalidated Chi-square analyses.

Paternity exclusion was determined using 99% CL of D scores. We calculated mean band-sharing values for female/chick couplets with no novel bands and used the lower 99% CL to establish acceptable levels for parentage (Emlen et al. 1998).

Results

Paternity analyses

For Jeffreys 33.15 and M13 probes combined, there were 22–90 (mean 51.1±13.5 SD, n=260) scorable bands on the DNA fingerprints of an individual. Mean band-sharing values for female/chick couplets with no novel bands were 0.61±0.08 SD (n=93), and the lower 99% confidence limit (CL) was D=0.42.

We found evidence that 4 of 232 offspring examined (1.7%) excluded the incubating male (Fig. 1). These include one offspring each from four clutches, representing 6.4% of all clutches (n=63). Band-sharing coefficients between these four chicks and incubating males fell below the lower 99% CL of the parent-offspring distribution (Fig. 1). All four came from nests where both putative parents were sampled, and these chicks also had substantially more novel bands than other chicks (Fig. 1). There was no evidence for intraspecific nest parasitism, since no chick fell below the lower 99% CL for D with the putative mother or had an unusual number of novel bands (Fig. 1). There was no evidence that any eggs were related to the male, but not female, parent.

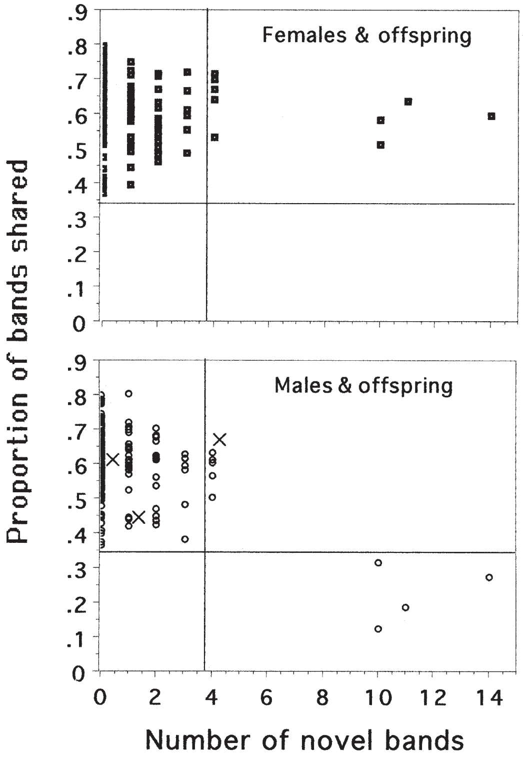

Fig. 1 Band-sharing coefficients between adult and offspring red-necked phalaropes (*Phalaropus lobatus*): *upper* adult females and young, including EPF young; *lower* adult males and young, including EPF young compared to incubating males and the first male (■) of the female. *Horizontal line* lower 99% CL for band-sharing between related individuals; *vertical line* upper 99% CL for novel bands

No offspring was excluded in 25 first clutches laid by females. Three of the four excluded offspring came from the six polyandrous clutches (50% of polyandrous clutches), one chick in each of three clutches. We were unable to exclude the previous mate of the polyandrous female as the father of each of these three chicks, since band-sharing coefficients with each offspring and that male were above the lower 99% CL and there were not unusually high numbers of novel bands (Fig. 1). The fourth exclusion occurred in 1 of the 16 same-pair replacement clutches. In this case, the female had not been seen associating with the incubating male for 4–5 days prior to nest loss and pair reuniting. No exclusions were found in the 16 nests of unknown status. Exclusions of the incubating male were more frequent in polyandrous than in monogamous (first and same-pair replacement) clutches (Fisher's Exact Test, P=0.015).

To estimate exclusion rates at the population level, we considered whether our sample of nests represented the population as a whole. Our sample included 53% first (n=25 clutches), 34% replacement (n=16 clutches), and 13% polyandrous clutches (n=6 clutches) among nests of known status. Each year of the study, 5–16% of females were polyandrous, with an annual mean of 9% (n=8 years) (Schamel 2000). Overall, 14% of females (n=47 females) were polyandrous in at least 1 year at the site (Rubega et al. 2000). Since some polyandrous females produce more than one polyandrous clutch (Schamel and Tracy, unpublished data), our sample probably slightly under-represents the frequency of polyandrous clutches. The frequency of replacement clutches varied annually from 8 to 42%, with a mean of 28% (n=307 clutches) (Schamel 2000). Our sample of 34% thus slightly over-represents such nests. Taking clutch sample bias into account, we estimate that 7% of clutches would contain an exclusion.

There was no obvious relationship between nests with exclusions and the time since association with the previous mate (6.0 days, range 4–9 for clutches with exclusions; 5.7 days, range 4–8 for polyandrous clutches containing no exclusions).

Mate guarding

Phalarope pair members remained within 5 m of each other most of the time throughout the laying period (overall mean=73.9±2.0% of time, n=81 pairs). Distances did not vary significantly among pairs or across laying stages (Fig. 2) (repeated measures ANOVA, $F_{9,179}$=0.83, P=0.58). The proximity of the pair to each other tended to arise from male movements (Fig. 3). Males were more likely to approach females than vice-versa (Fig. 3); both males and females spent more time approaching each other than moving away (repeated measures one-way ANOVA of movement categories, $F_{3,176}$=73.87,

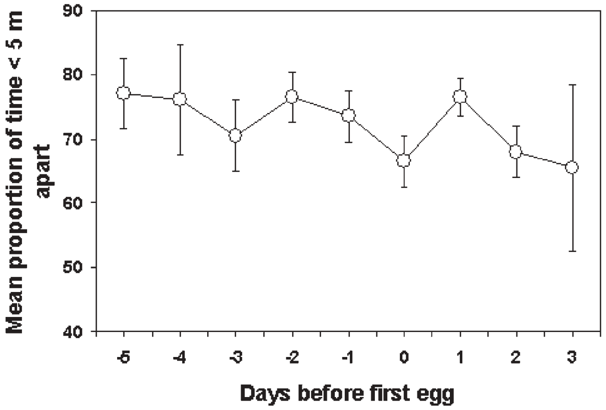

Fig. 2 Proportion of time red-necked phalarope pair members were within 5 m. Mean±SE, day 0 = first egg laid

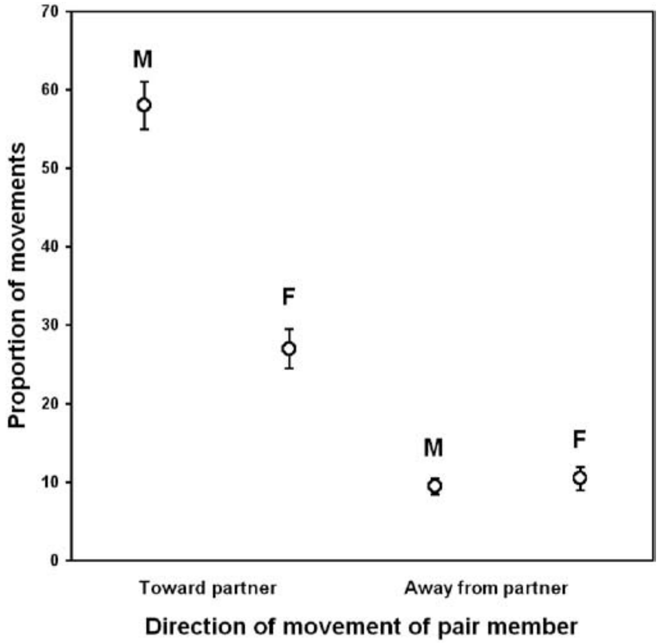

Fig. 3 Proportion of observations in which red-necked phalarope pair member moved toward or away from partner. Mean±SE

$P<0.0001$, $n=45$ pairs; Bonferroni $P<0.05$ for each significant contrast). Females and second males stayed within 5 m more often than first/renesting pairs (*t*-test, $t_{43}=2.43$, $P=0.02$; mean=81.8±2.9%, $n=18$, versus 72.5± 2.4%, $n=63$).

Within-pair copulations

We recorded 178 successful (mean=0.55±0.08/h) and 187 unsuccessful (mean=0.63±0.07/h) within-pair copulations during focal pair observations ($n=227$ days with observations). Successful copulation rates varied significantly by laying stage (Fig. 4a; one-way ANOVA, $F_{7,194}=2.07$, $P=0.048$). Laying stage –2 differed significantly from laying stages –3, 1, 2, 3 (Bonferroni adjustment; $P<0.05$); laying stage 3 differed significantly from laying stage –1 and 0 (Bonferroni adjustment; $P<0.05$).

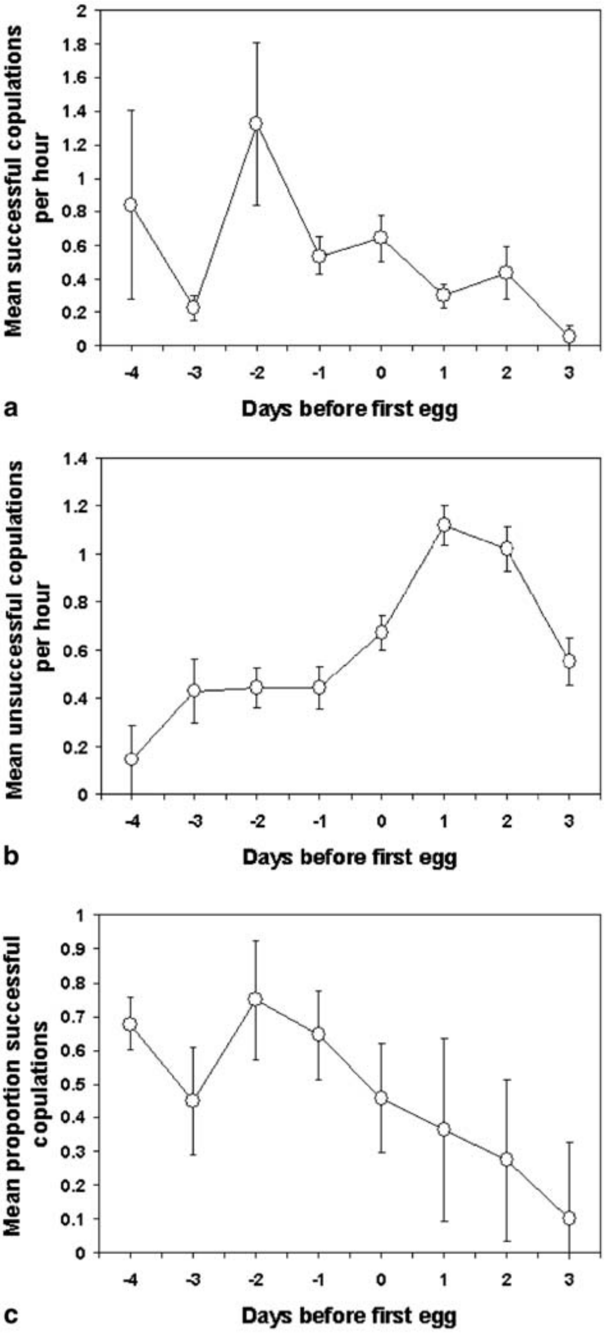

Fig. 4a–c Patterns of within-pair copulation of red-necked phalaropes, with respect to laying stage. **a** Rate of successful copulation, **b** rate of unsuccessful copulation, **c** proportion of attempts that were successful. In all panels, the mean±SE is shown

Successful copulation rates varied among known clutch types (first, polyandrous, replacement), being higher in renesting pairs than either first (one-way ANOVA, $F_{2,152}=2.98$, $P=0.05$; contrasts test first versus renest, $F=4.96$, $P=0.03$) or polyandrous pairs (contrasts test first vs second, $F=3.89$, $P=0.05$). Unsuccessful copulation rates did not differ by clutch type (one-way ANOVA, $F_{2,152}=2.38$, $P=0.10$). Unsuccessful copulation rates varied significantly by laying stage (Fig. 4b; one-way ANOVA, $F_{7,194}=2.43$, $P=0.02$), being significantly higher during the production of eggs 2 and 3 than during prelaying or the production of the first egg (Bonferroni adjustment; $P<0.05$). The percentage of copulations that were successful varied by laying stage (Fig. 4c; repeated measures ANOVA, $F_{7,130}=3.56$, $P=0.001$); percent successful copulations were lower during the production of eggs 2–4 than during the 2 days prior to the production of the first egg (Bonferroni adjustment; all comparisons $P<0.05$).

Extra-pair copulations

We recorded seven successful EPCs during 337.7 h of focal pair observations (0.02/h) and 53 unsuccessful EPCs (0.16/h). None of these copulations involved previous mates. With-in pair copulation attempts were thus substantially more likely to be successful than extra-pair attempts (48%, $n=365$ copulation attempts vs 12%, $n=60$ copulation attempts, $X^2=27.8$, $P<0.0001$). Attempted EPCs occurred throughout the nesting season (mean=16.6±1.0 days after the first egg in the population; range=30 May–27 June), but successful EPCs occurred only after the middle of the season (mean=17.4 days after the first egg in the population; range=11–22 June). Unsuccssful EPCs came primarily from paired, but prelaying, or incubating males (73.6% paired/prelaying; 9.4% incubating; 17.0% unknown status) whose own nests were from 4 days before laying to early incubation. All successful EPCs from males occurred after the male had received the first egg of his clutch. Unsuccessful EPCs focused primarily on paired/laying or post-laying females (54.7% paired/laying; 7.5% post-laying; 37.8% unknown status) from 3 days prior to laying the first egg in a clutch to early post-laying, but primarily during egg-laying. Successful EPCs occurred during late laying.

Infertile eggs

Because we collected eggs for the study, we were able to estimate the infertility rate directly. Five clutches contained one infertile egg each (7.9% of the clutches, $n=63$ clutches). Of the 245 total eggs in this study, 2.0% were infertile. Some eggs were obviously fertile, but did not yield ample DNA for inclusion in the paternity analysis.

We knew the clutch status for four of the five clutches containing an infertile egg; all four were first clutches. First clutches were more likely to contain infertile eggs than second clutches (Fisher's Exact Test, $P=0.043$). Polyandrous females were not more likely to produce infertile eggs in their first clutches than monogamous females (Fisher's Exact Test, $P=0.068$). Unfortunately, we did not know the laying order of eggs in any clutch containing infertile eggs.

Discussion

As predicted for a species in which males provide all parental care (Birkhead and Møller 1992; Møller 2000; Griffith et al. 2002), paternity by incubating male red-necked phalaropes was very high (98.3% of chicks; 93.6% of clutches). The few cases of exclusion were consistent with our understanding of sperm competition (Birkhead and Møller 1992; Birkhead and Fletcher 1995; Colegrave et al. 1995; Birkhead et al. 1999); they all occurred in second clutches (50% of polyandrous clutches, and 6% of same-pair replacement clutches). We were unable to exclude the first male as the father in the cases found in polyandrous clutches (Fig. 1), and given the high probability of doing so if he did not sire the offspring, we conclude that this male was the true father. We rarely observed successful copulations between the female and her first male following the deposition of the second egg and we never saw successful copulations between incubating males and their former mates. Thus, paternity by the first mate likely arose from stored sperm, as in spotted sandpipers (Oring et al. 1992), rather than from ongoing copulations with the first male following clutch completion, as in wattled jacanas (Emlen et al. 1998).

Close association with females (Pierce and Lifjeld 1998), mate following (Owens et al. 1995; Komdeur et al. 1999; Blomqvist et al. 2002), frequent copulations (Birkhead and Møller 1992), and strategic timing of copulations (Owens et al. 1995; Mee et al. 2004) have been successful tactics by males for paternity assurance in other species. These tactics appear to be used by male phalaropes as well. Second males guarded females more strongly than first males, consistent with the increasing threat of EPCs by the growing number of incubating males as the breeding season progressed. Pair members in red-necked phalaropes remained closer during prelaying and laying than the few other shorebird species where mate-guarding has been reported (Oring et al. 1994; Pierce and Lifjeld 1998). Closer associations, coupled with mate guarding, might help prevent EPFs. However, neither of these tactics reduces the fertilization risk by stored sperm from a previous mate.

The frequency and timing of copulation may protect a male against fertilizations from stored sperm. As predicated, red-necked phalaropes had a relatively high copulation rate (0.55 successful copulations/h). Other shorebirds range from a low of 0.034/h for purple sandpipers (*Calidris maritima*) (Pierce and Lifjeld 1998) to 0.44/h for semipalmated plovers (*C. pusilla*) (Zharikov and Nol 2000), both territorial species. The rate we reported is surpassed only by the territorial, simultaneously polyandrous wattled jacanas, where co-mates are an ongoing EPP threat and females actively solicit copulations from non-incubating mates; male jacanas apparently defend against EPP by the extraordinary copulation rate of 1.3/h (Emlen et al. 1998), although this figure includes copulations by co-mates.

We found evidence for strategic timing of copulations. Both the percentage of copulations that were successful and the successful copulation rate were significantly higher during late prelaying than during late laying. Such success also suggests that females usually cooperate with the incubating male in his attempts to fertilize the current clutch. We could not detect whether the three females that did fertilize an egg with a previous mate's sperm behaved any differently than other females.

Although mates appear to cooperate in some instances (see above), the timing of unsuccessful copulations suggests sexual conflict. Unsuccessful copulation rates were highest after the production of egg 2 (Fig. 4b), as was the percentage of unsuccessful copulations (Fig. 4c). Taken together, these figures provide information on the total copulation effort and the relative copulation success. These data strongly suggest that males are attempting to deposit sperm that could fertilize subsequent clutches of their female, especially through copulation attempts on or after day 2 (after the deposition of egg 3) (Fig. 4b,c); the last egg in the clutch will have already been fertilized at that time. Females clearly resist such copulation attempts (Fig. 4b). One interpretation is that the female faces a trade-off between fertilization of the current clutch versus the potential for obtaining a second mate. Natural selection should produce males that can detect females carrying stored sperm, and reject those females as potential mates in favor of females not carrying stored sperm (Whitfield 1990). Cessation of copulations as soon as possible after enough sperm has been received to fertilize the current clutch could improve a female's likelihood of obtaining a second mate. This tactic carries a risk of halting copulations too early, which could result in infertile eggs, especially later eggs in first clutches. Second clutches should be more likely to have fertile eggs due to stored sperm, combined with sperm from the second male (Wetton and Parkin 1991). We found a significantly higher infertility rate in first versus second clutches. Unfortunately, we were unable assess if egg order was related to infertility.

Although we failed to show a significant difference in infertility in first clutches between monogamous and polyandrous females (P=0.068), infertile eggs may have been more common in first clutches of polyandrous (2 of 6 clutches) than monogamous (2 of 41 clutches) females. Our analysis was hindered by a small sample size of polyandrous females. We encourage others to examine the relationship between infertiltiy in first clutches and a female's ability to acquire a second mate.

Although we predicted elevated copulation rates in second clutches compared to first clutches (Birkhead and Møller 1992), we found this only in replacement clutches of monogamous pairs and not in polyandrous pairs. Replacement nesting is common following nest failure. There appears to be strong selection pressure to retain original mates; only 6% (n=46) of all renesting males changed mates when their original female was available (Schamel et al. 2004). We observed two males immediately after collecting eggs from their incubated clutches. After a brief interlude of repeatedly returning to the nest, then preening, they flew directly to the location where their former mate was feeding and began immediate courtship (Schamel and Tracy, unpublished), as also occurred immediately following nest loss in spotted sandpipers (Lank and Oring, unpublished). Schamel et al. (2004) argued that repairing with the same female, as opposed to pairing with an unknown female, decreased the male's probability of obtaining eggs with foreign sperm in his second clutch. Nonetheless, his first mate may already have remated and be carrying stored sperm in addition to his own. One renest in this study contained a non-paternal egg, and frequent copulation will be in the male's interest. From the female point of view, whatever factors led to the initial choice of that male for the first clutch probably still exist, and monogamy reduces the risk of sexually transmitted disease (Lombardo 1998; Westneat and Rambo 2000). Since forced copulations have never been reported for phalaropes (Delehanty et al. 1998; Dale et al. 1999; Rubega et al. 2000), the high copulation rate in repairing birds is most likely related to providing paternity assurance for the male by the female.

Surprisingly, an elevated rate of successful copulation was not observed in polyandrous pairs. In our population, females became polyandrous through pairing with renesting males whose original females were not available (76.5%; n=18 males; Schamel et al. 2004) and with newly arriving males (23.5%). Selection should favor females that can hide their status as having already produced a clutch that season (Whitfield 1990). The lack of a particularly high copulation rate in this situation would be consistent with females being successfully able to do so. Males formed pair bonds with these females at the same rate as with females that had not previously produced a clutch (Schamel et al. 2004).

Our data suggest that male phalaropes pursue a mixed reproductive strategy. First, they attempt within-pair copulations late in egg laying, much later than required for fertilization of the current clutch (Fig. 4b). In fact, the unsuccessful copulation rate at the laying of the last egg is higher than the successful copulation rate of most shorebirds (Pierce and Lifjeld 1998; Delehanty et al. 1998; Oring et al. 1994; Zharikov and Nol 2000; Mee et al. 2004). Second, pre-incubation and early incubating males attempt EPCs with females seeking a mate, as seen in spotted sandpipers (Colwell and Oring 1989). A small percentage of these result in cloacal contact, and we present indirect evidence that such EPCs occasionally result in EPP. The one EPP implicated by this means occurred at the peak of EPC risk in our population, when many males were paired and/or beginning incubation. Third, if fertilization of second clutches through stored sperm is a tactic of first mates, first males should not impede rapid mate switching. Indeed, agonistic behaviour was never observed between a current male and a male attempting to form a pair bond with the female immediately after deposition of the penultimate egg (Schamel and Tracy 1977; unpublished data).

Our results also help interpret previously published results of paternity in red and Wilson's phalaropes. Given the absence of observed EPCs and the concentration of EPP in later clutches, red phalaropes are thought to achieve EPP through stored sperm from a prior male. Data presented here strengthen that conclusion. No EPP at all was detected in Wilson's phalaropes (Delehanty et al. 1998), probably because all but one of the nests sampled were first clutches, and the single additional clutch examined was a replacement clutch. A comparable sample from red-necked phalaropes would likely have produced exactly the same result. EPP in Wilson's phalaropes should be re-examined.

In conclusion, we suggest that EPP patterns in phalaropes is best explained as a three player interaction, as suggested by Westneat and Stewart (2003). Males appear to pursue a mixed reproductive strategy. To provide their sperm for the current clutch, they have high copulation rates that peak one day prior to fertilization of the first egg, and they perform mate guarding. Strategies to inseminate other clutches include high rates of attempted copulations late in the laying stage, stored sperm, facilitation of female re-pairing, and EPCs with pair-seeking females. Females facilitate paternity assurance of second mates by rejecting within-pair copulations late in egg laying, which should reduce sperm competition for future mates, and by rejecting copulations by potential future mates until late in laying of the current clutch. Females apparently incur an infertility cost by rejecting some within-pair copulations associated with their first clutches. Most EPP occurred in polyandrous clutches, and second males are apparently unable to detect prior pairings of females. The stability of this polyandrous system, in which males provide all parental care, ultimately may depend on females providing males with eggs containing primarily genes of the incubating male, and not a previous mate.

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