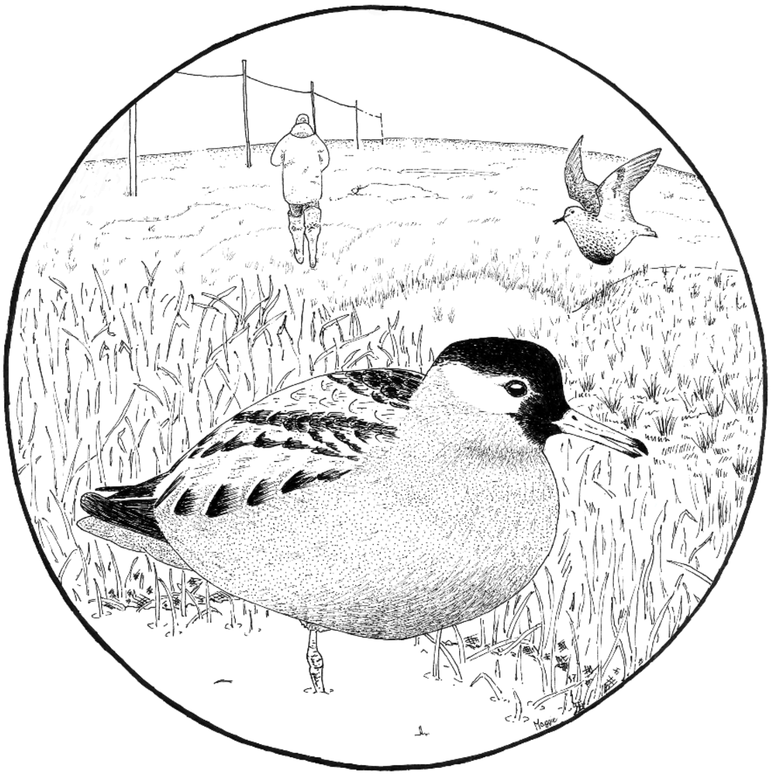
Mating behaviour of two polygamous shorebird species in the Arctic



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For my parents Karin and Martin Krietsch, which always supported me to follow my own interest and at all times were there for me along the way.

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Summary

The mating behaviour is a critical part of an individual's life, given that it directly influences the reproductive success of sexually reproducing organisms. In nature, we can observe a fascinating diversity of mating strategies shaped by sexual and natural selection. Understanding the evolution of this diversity is a central part of evolutionary biology. Nevertheless, the underlying evolutionary and ecological factors that shape the evolution of this diversity are still not fully understood, and on top of that we even lack detailed knowledge of the mating behaviour for many species.

In this dissertation, we enhance our understanding of the mating behaviours and strategies of two polygamous shorebird species. The focus of our studies are the polyandrous red phalarope *Phalaropus fulicarius* and the polygynous pectoral sandpiper *Calidris melanotos*, two sympatric breeders of the Arctic tundra with distinctly different mating systems. More specifically, we investigated the social and genetic mating system of the red phalarope, by quantifying the number of social and genetic mates and describing the copulation behaviour in this context. This allowed us to test the "sperm storage hypothesis", which predicts that extra-pair paternity in sequentially polyandrous species is mainly the result of sperm stored by females from within-pair copulations with a previous mate. Next, we investigated mate guarding behavior in red phalaropes under consideration of the male and female perspective and in relation to breeding phenology, time relative to mean clutch initiation, and to other mutually exclusive behaviours, like incubation or mate searching. Furthermore, we investigated if the apparently nomadic movements of pectoral sandpipers between potential breeding sites are influenced by the prevailing wind conditions. Finally, I describe the mating system of both species in detail, discuss how sexual selection shapes the mating strategies in both species and sexes, and discuss how environmental conditions influence mating strategies.

To accomplish this, we recorded in great detail the mating behaviour of red phalaropes during the time span of three breeding seasons in Utqiaġvik, Alaska. We caught and colour-banded nearly all individuals and documented all breeding attempts within our study site. Eggs were collected and artificially incubated to prevent data loss due to predation events, which allowed us to perform comprehensive paternity analysis. Furthermore, modern tracking devices allowed us to follow individual movements and pair-wise association patterns continuously, which allowed us to study variation in mate guarding behaviour. Additionally, we used a previously published data set of pectoral sandpiper movements from two breeding season, in combination with wind data from a global reanalysis model to investigate the influence of wind conditions on breeding site sampling behaviour.

We found lower rates of social polyandry and genetic polyandry in red phalaropes than previous studies reported. Overall, 7% of females (11/162) had multiple social mates and extra-pair paternity occurred in 11% (37/334) of the nests. Our paternity analysis and behavioural observations provide limited evidence for the sperm storage hypothesis. Our findings indicate that stored sperm from a previous mate does not significantly contribute to extra-pair paternity in this sequentially polyandrous species. Instead, extra-pair paternity was generally due to two mechanisms: firstly to extra-pair copulations by both sexes during the period between pair establishment and early incubation; then to rapid mate switching by females in the context of attempts to acquire multiple care-giving males. We show that red phalarope pairs were almost continuously together in the days before clutch initiation and showed no sex-bias in separation movements, which suggests that both pair members guard their mate. Still, limited sexual conflict arises through biases in the operational sex-ratio and a trade-off with male nest attendance. We found no clear relationship between mate guarding intensity and the occurrence of extra-pair paternity. Our analysis on the breeding site sampling behaviour of pectoral sandpipers suggests that the wind conditions influence movements in two ways. First, stronger wind support led to increased ground speed and was associated with a longer flight range, and second, males had a higher chance of flying in the direction with more favourable wind conditions.

In conclusion, we found that extra-pair paternity in red phalaropes can mainly be explained by female strategies to acquire multiple mates and that in this non-territorial socially polyandrous species, mutual benefits of mate guarding might be the process underlying the evolution of a brief but strong social pair bond, with the unique purpose of producing a clutch for a care-giving male. Polygamy in both species is likely influenced by the length of the breeding season and its spatiotemporal variation throughout the breeding range, as well as local operational sex ratios. Large scale breeding site sampling behaviour can be influenced by the prevailing wind conditions in pectoral sandpipers and consequently effect local breeding densities. Both species are characterised by strong intrasexual selection and direct fitness benefits seem to play a more important role than indirect fitness benefits in mate choice. Still, it is necessary to consider the interplay of intrasexual selection and mate choice to completely understand the factors shaping sexual selection, especially in the context of sex-specific biases in the operational sex ratio and individual strategies to maximise reproductive success from the male and female perspective.

General introduction

In sexual reproduction, a new generation of individuals is created by the fusion of haploid gametes. In unicellular organisms, the gametes were equal in size (isogamy), but, in multicellular eukaryotes, the competition over fertilization led to the evolution of size dimorphism in gametes (anisogamy; Otto and Lenormand, 2002; Parker et al., 1972). The size difference observed at the level of the gametes defines the biological sexes (Goymann et al., 2023): In females they are few in number, larger and typically non-mobile (egg cells), whereas in males they are numerous, smaller and often mobile (sperm or pollen). Anisogamy, is almost universal in complex multicellular eukaryotes (Bell, 1978) and it creates an imbalance between the sexes, as females are limited to produce fewer and energetically more costly larger eggs, compared to males that produce many smaller and comparatively energetically cheaper gametes. This unbalance is an important factor in the evolution of "typical" sex roles, which are characterized by higher male-male competition than female-female competition, and greater or exclusive female care (Schärer et al., 2012; Trivers, 1972). The resulting evolution of traits related to mating and reproductive success of sexually reproducing organism was recognised by Darwin in *The Origin of Species* (1859) and further developed in *The Descent of Man and Selection in Relation to Sex* (1871) under the concept of sexual selection, which has been further refined ever since (Hoquet, 2015).

While natural selection describes how certain heritable traits (or individual units of DNA) become more or less common in a population over time, because of a higher probability of survival in interaction with the environment, sexual selection describes "*the advantage which certain individuals have over other individuals of the same sex and species, in exclusive relation to reproduction*" (Darwin, 1871). In other words, sexual selection arises from differences in mating success that can be thought of as intraspecific reproductive competition (Hosken and House, 2011). Sexual selection can be partitioned into two mechanisms: intrasexual selection and intersexual selection.

Intrasexual selection, the competition between individuals of the same sex (typically males) for access to mates, can take various forms and may involve direct physical combat and displays of strength or dominance In some species this leads to territoriality or mate monopolisation, but may also happen after copulation in the form of sperm competition. In contrast, intersexual selection, also known as mate choice, occurs when individuals of one sex (typically females) choose mates based on certain traits. Mate choice can be based on female (or male) preferences for physical traits (e.g. size, coloration or symmetry), elaborate courtship displays, provided resources or parental investment, compatibility between mates, or be a passive process based on the behaviour of the individual. For example, if females of a species only mate on top of specific mountains, there will be selection against males that do not visit these mountains. To understand sexual selection, it is essential to consider the interplay of both mechanisms (intra- and intersexual selection) together (Hunt et al., 2009) and to consider the male and female perspectives, since the two sexes are subject to different selective pressures. This interplay created the fascinating and diverse variation in mating patterns, parental care and energetic allocations to progeny we can observe today (Shuster, 2009).

Animal mating systems

This variation in reproductive behaviour is classified into mating systems. In animals, mating systems are typically defined based on the number of mates acquired, the strategy of mate acquisition, the presence and characteristics of a pair bond, and investment in parental care by each sex (Emlen and Oring, 1977). The most basic distinction is between monogamy and polygamy (which is an umbrella term for all non-monogamous mating systems). Monogamous mating systems are characterized by the exclusive relationship between one male and one female, which form a pair bond that typically persist for a relatively long time (i.e. for multiple reproductive cycles) or a life time (Bales et al., 2021). On top of that, monogamous species often have biparental care. In contrast to this, species that exhibit a polygamous mating system are characterised by individuals that mate with multiple individuals of the opposite sex. Three general types can be distinguished: Polygyny, where one male mates with multiple females, polyandry, where one female mates with multiple males, and polygynandry, where both males and females have multiple mating partners.

Based on these classifications, the number of mates typically refers to a single reproductive cycle (breeding season) and can be further characterized by considering temporal aspects of mating. Polyandrous females can, for example, mate with multiple males either simultaneously or sequentially. Species and populations are typically classified in terms of these mating systems based on a proportion of individuals engaging in certain strategies, but it is important to point out that there can be significant variation in mating strategies within species and populations (Johnson and Burley, 1998). One classic example for this is the dunnock (*Prunella modularis*), in which females are most commonly polyandrous, but individuals can also have monogamous, polygynous or even polygynandrous relations (Davies and Lundberg, 1984).

To understand the evolution of this variation in mating systems, it is important to consider the potential of males or females to monopolize mates, which allows more detailed classifications of polygamous mating systems (Emlen and Oring, 1977). This potential is dependent on environmental and phylogenetic constraints in parental care, and the spatiotemporal distribution of resources and ultimately of receptive mates (Emlen and Oring, 1977). For example, polygyny is widespread in mammals due to the physiological constraints that females face regarding parental care, which in turn sets a high potential for male monopolization (Clutton-Brock, 1989). In contrast, in birds, females lay undeveloped eggs that typically require constant care (i.e. biparental care) and social monogamy is consequently most common (Johnson and Burley, 1998; Kempenaers, 2022).

The clumped distribution of resources can allow males to monopolize females either indirectly by limiting access to competitors (resource defence polygyny), or directly by defending females that aggregate for environmental reasons (harem). When resources or mates cannot be monopolized by males, they can form leks, which are aggregations of males that engage in competitive displays and courtship behaviour. Females then visit these leks, copulate with one or multiple mates and afterwards usually provide uniparental care. Hence, lekking males can mate polygynously while being sure that the females will care for the offspring. In contrast, when a female mates polyandrously she has to at least form a short-term pair bond that lasts at least until egg-laying (female access polyandry) or directly defend a territory with male sub-territories (resource defence polyandry; Emlen and Oring, 1977) in order to ensure male care for her offspring.

Further complexity to mating patterns is given by the spatiotemporal distribution of receptive mates, which is conceptualized as the operational sex ratio (Kokko et al., 2012; Kvarnemo and Ahnesjo, 1996). The operational sex ratio, defined as "*the average ratio of fertilizable females to sexually active males at any given time*" (Emlen and Oring, 1977), influences the strength of sexual selection and determines the potential to monopolize mates. If the operational sex ratio is skewed towards females (i.e. excess of females), the potential for males to become polygynous increases, and if the ratio is skewed towards males, then polyandry is more likely (Emlen and Oring, 1977). Particularly in species with flexible mating systems, the operational sex ratio is the best predictor of the mating strategy of individuals and therefore of the mating system of a population.

These general classifications of mating systems are typically based on social associations of individuals. The development of modern DNA fingerprinting methods (Burke and Bruford, 1987) added another perspective to mating system definitions and it revealed that genetic polyandry (and polygyny) is wide spread. For example, while around 80% of birds are socially monogamous, in more than 75% of them some individuals engage in copulations outside their social pair bond (extra-pair copulations) that lead to extra-pair paternity (Brouwer and Griffith, 2019; Cockburn, 2006; Griffith et al., 2002). Similar to the variation in social mating patterns, the frequency of extra-pair paternity can vary greatly between species, populations and even among individuals of the same population (Brouwer and Griffith, 2019; Petrie and Kempenaers, 1998; Valcu et al., 2021). While the benefits of extra-pair paternity are easy to understand from the male perspective, as a direct increase of their reproductive success, the benefits for females are still debated (reviewed in Forstmeier et al., 2014).

Birds naturally went on the forefront of mating system research, since they show almost all the variety of social and genetic mating systems, are comparably easy to observe, and their reproductive success can be accurately measured (Kempenaers, 2022). Particularly shorebirds (Charadriiformes) are an optimal study system for mating system evolution, as they show a wide continuum of types of parental care (biparental or uniparental by males or females) and mating behaviours (Thomas et al., 2007). Shorebird mating systems range from polygyny with female only care (e.g. lekking: ruff *Calidris pugnax*, buff-breasted sandpiper *Calidris subruficollis*, great snipe *Gallinago media*; territorial: pectoral sandpiper *Calidris melanotos*), through monogamy with biparental care (many plovers and oystercatchers), to classical polyandry with male only care (e.g. sequential / female access polyandry: phalaropes *Phalaropus* spp.; simultaneous / resource defence polyandry: wattled jacana *Jacana jacana*, comb-crested jacana *Irediparra gallinacea*). Moreover, there are species with more flexible parental care types, which allows for mating system variation within species and populations (e.g. biparental incubation and potential for uniparental brood care by both sexes: Kentish plover *Charadrius alexandrinus*, snowy plover *Charadrius nivosus*; split clutches between sexes with uniparental incubation and care: mountain plover *Charadrius montanus*; uniparental or biparental incubation and care: sanderling *Calidris alba*). This diversity of mating behaviours makes it challenging to apply gerneral applicable definitions.

Sex-role reversal and polyandry – The exception that confirms the rule?

In few animal species, “typical” sex roles are reversed, such that females compete more strongly for mating opportunities than males, and males provide more or all parental care. In birds, only less than 1% of the species have male-only care, while in contrast 5-10% of the species have female-only care (Reynolds et al., 2002). Sex-role reversal is often combined with classical polyandry, a term coined by Oring (1986) to distinguish simultaneous and sequential polyandry from cooperatively polyandrous species. The evolution of sex-role reversal already puzzled Darwin (1871) and, despite fruitful research progress since then, our understanding of which ecological, phylogenetic and social factors facilitated conventional sex roles to become reversed multiple times is still limited (Andersson, 2004; Ligon, 1993; Liker et al., 2013; Oring, 1986; Safari and Goymann, 2020).

One foundation for the possibility of sex-role reversal in species with post-zygotic parental care (like birds), is likely a food rich environment that allows successful reproduction with uniparental care (Andersson, 2005; Erckmann, 1983). The reduced necessity for parental care can further promote the transition to uniparental care. For instance, almost all classical polyandrous bird species are within the shorebirds and have precocial young, that leave the nest within hours after hatching, feed themselves and are only dependent on the parents for guidance, brooding and defence against predators (Thomas et al., 2007). The only known exception in birds is the black coucal *Centropus grillii*, that has altricial young that are underdeveloped at hatching (Goymann et al., 2004). A further hypothesis for the evolution of male-only care, is that when the breeding season is short and nest predation rates are high, females could benefit from the potential to produce multiple clutches to quickly replace a lost clutch (Andersson, 2005; Erckmann, 1983). This could have initially promoted reduced female care and ultimately male-only care, since females freed from providing care can more quickly gather the resources to lay another clutch.

However, these phylogenetic and environmental circumstances alone are not enough to explain the evolution of sex-role reversal and classical polyandry. An example can be found in the shorebird species that often breed sympatrically in the Arctic tundra, most of which are still socially monogamous and biparental despite breeding in the same environment and experiencing similar predation rates and food conditions (Thomas et al., 2007). Nevertheless, some of these biparental species seem to have the potential for uniparental care, which can be observed when one of the parents goes missing (Bulla et al., 2017), and also have the potential to lay replacement clutches (Naves et al., 2008). So, what prevents them from evolving uniparental care? The simple answer is that the reproductive success of females (or males) that do not provide care has to be higher than of those that do provide care. If there is no potential for females (or males) to have multiple mates within one breeding season, monogamy with biparental care is likely the strategy that brings the highest reproductive success (Kokko and Jennions, 2008). Only when some individuals manage to access multiple mates, then polygamy becomes advantageous and these individuals will have a higher reproductive success (Emlen and Oring, 1977; Safari and Goymann, 2020).

One scenario in which individuals from one sex can potentially access multiple mates, is when the operational sex ratio is biased (Emlen and Oring, 1977). An interesting insight into this hypothesis is offered by those species that show flexible parental care, where in fact the operational sex ratio influences sex roles, with the rarer sex deserting more often and having a higher prospect to find an additional mate (Carmona-Isunza et al., 2017; Kupán et al., 2021; Reneerkens et al., 2014; Thomas and Székely, 2005). These biases have often been linked to the adult sex ratio, defined as the number of adult males to females in a population, which indeed is related to sex roles and polygamy (Kappeler et al., 2023; Kokko and Jennions, 2008; Liker et al., 2013; Székely et al., 2014). While we start to understand how these biases in adult sex ratios can be maintained by sex-specific differences in survival (Eberhart-Phillips et al., 2017; Veran and Beissinger, 2009), recent theoretical work points to the importance of considering how mortality relates to care and competition, which both affect lifespan and reproductive success (i.e. sex ratio at maturation; Fromhage and Jennions, 2016; Jennions and Fromhage, 2017), which further complicates the picture for the empiricists.

In practice and in the wild, it is often really difficult, if not even impossible, to get valid and reliable estimates for the different types of sex ratios (adult or operational sex ratio, or sex ratio at maturation), since they are often linked to sex-specific differences in movements and detectability (Kempenaers, 2022). Nevertheless, they are a key element in understanding the evolution of sex-role reversal and sex roles in general (reviewed in Kappeler et al., 2023; and Safari and Goymann, 2020).

Mate searching and mate choice

The evolution of sexual reproduction created the necessity to find, and the possibility to choose among mates. Mate searching behaviour is an essential part of mate choice as it influences mate-encounter rates and therefore the pool of mates that can be chosen from. In birds, mate searching strategies usually focus on mate attraction, via advertisement calls or elaborate displays, and on active movements. Both strategies, attracting and moving, are usually linked to habitat quality. High-quality habitats attract more mates on one hand, but on the other one they increase local intrasexual competition. This can be observed, for example, in the relationship between territory size and quality, typically with territories being smaller in high-quality habitats (Adams, 2001). Since mates are ultimately a resource that is dependent on food, it is also not a surprise that first models of optimal mate searching resemble those of optimal foraging theory, in which males try to reach optimal mating probabilities by balancing the costs and benefits of mate searching (Parker, 1978).

Males are typically the sex that invests more in mate searching behaviour, as they are usually able to gain higher reproductive success via additional mate encounters than females (see above section on animal mating systems). These sex differences are likely linked to different investment into parental care and consequently different “mating windows” (defined for females as the time between receiving the first sperm that can fertilize the eggs and the latest insemination point; and for males as the time from one copulation until the sperm supply is replenished) (Fromhage et al., 2016). Consequently, the timing of mating matters more for males, which theoretically could fertilize different females with each ejaculate, compared to females that are constrained to how much offspring they produce and that have a longer period in which they can receive the sperm necessary for fertilization. Females can further extend their mating window by storing sperm (Holt and Fazeli, 2016), which further reduces the time pressure on mate searching (Fromhage et al., 2016). However, in low densities, females may nevertheless be limited by mate availability and may need to invest in active searching behaviour even in species with “typical” sex roles (Kokko and Rankin, 2006). In contrast, in sex role reversed species, where males are the limiting sex, females are the ones generally expected to invest more in mate searching.

If individuals encounter multiple mates within the reproductive window, they can be choosy. Again, typical sex roles predict that the limiting sex (typically females) should be choosier than the limited one. While there is overwhelming evidence that non-random mate choice exits, we are still lacking a clear picture of the rules that are in place (Ryan et al., 2007). One emerging pattern is that direct benefits are likely more important than indirect benefits (Kirkpatrick and Barton, 1997; Kokko et al., 2003). Direct benefits increase the fitness of the choosy sex by directly providing material advantages like high-quality territories, nutrition, parental care or protection, whereas indirect benefits increase the quality of the offspring (Fisherian runaway, “good genes”, or “genetic compatibility”) (reviewed in Andersson and Simmons, 2006; Hosken and House, 2011). However, mate choice may also happen in the absence of any benefits to the chooser, for instance if the advertiser simply makes use of existing sensory biases in the receiver (“sensory exploitation”; see Arnqvist, 2006). It describes the coevolution of traits of the advertising sex to match the preferences of the choosy sex that initially evolved for other reasons like foraging preferences or predator avoidance. If the chooser does not happen to benefit from this, sensory exploitation often leads to sexual conflict and to the subsequent evolution of resistance to sensory exploitation (Holland and Rice, 1998).

In the last decades, it became apparent that sexual selection can also work post copulation in the form of sperm competition and “cryptic” female choice (Birkhead and Moller, 1992; Birkhead and Pizzari, 2002). For example, in sequentially polyandrous species it has been proposed that females store the sperm of the higher-quality first mates to fertilize the offspring of subsequent clutches with lower-quality males (Oring et al., 1992). Besides this, extra-pair paternity is widespread in socially monogamous bird species (Brouwer and Griffith, 2019). It seems clear then that “socially” monopolizing a female might not be enough to ensure paternity. Males also have to invest in strategies to “genetically” monopolize females, which typically translate into mate guarding strategies (Birkhead, 1981; Birkhead and Moller, 1992). Such defensive practices aim to limit female-mate encounter rates and, in consequence, can constrain female mate choice and genetic polyandry.

Polygamous species are particularly interesting to study in the context of mate searching and mate choice, as they are characterized by strong sexual selection (Emlen and Oring, 1977; Selander, 1965). In polygynous species, males are often free from parental care and therefore mainly invest in strategies to maximise the number of female encounters and fertilizations, while females have potentially a lot of freedom to choose a preferred mate. In polyandrous species, in contrast, females might invest more in mate searching and males might invest more in mate choice. The fundamental difference to polygynous species is that the mate searching sex (here the females) has to produce the offspring and that the providers of care (here the males) should get some assurance of paternity.

Study species

We studied two polygamous shorebird species: The red phalarope *Phalaropus fulicarius* and the pectoral sandpiper *Calidris melanotos* (Figure 1). Both species breed sympatrically throughout most of the high and low Arctic (Figure 2). While red phalaropes breed circumpolar, pectoral sandpipers' breeding range is limited to the Arctic coastal plains of east and central Siberia, Alaska and north-west Canada (Farmer et al., 2020; Tracy et al., 2020). Moreover, both species primarily use wet coastal habitat, characterised by marshy tundra scattered with low-lying ponds, often within a mosaic of raised hummocks. For nest sites, pectoral sandpipers prefer better drained sites like raised mounds or polygon edges (Pitelka, 1959), while red phalaropes typically breed in more moist areas, sometimes close to standing water or ponds (Kistchinski, 1975; Mayfield, 1979). After the breeding season, both species perform a long-distance migration and mainly winter in the Southern Hemisphere moving along very distinct routes. Pectoral sandpipers predominantly migrate over land to southern South America, and to a lesser extent southern Australia and New Zealand, and spend their nonbreeding period in habitats similar to the breeding grounds: marshy areas, grasslands, and wetland (Farmer et al., 2020). Red phalaropes, instead, switch to an entirely pelagic lifestyle once the breeding season is over, migrating in offshore waters, often in association with oceanic fronts. They then spend their nonbreeding period mainly along the Pacific Ocean coast from southern North America to southern South America, and in the Atlantic Ocean coast offshore west Africa and south-west Africa, as well as, in the western Atlantic Ocean at the edge of the Gulf Stream. Red phalaropes can therefore spend up to 11 months in marine habitats (Tracy et al., 2020) and are practically seabirds within the sandpiper family (Scolopacidae).

Figure 1. A pair of red phalaropes *Phalaropus fulicarius* (top) and a pectoral sandpiper *Calidris melanotos* male (bottom). Red phalaropes are sex-role reversed, with larger and more brightly plumaged females (top left) than males (top right). This pair is performing a "pushing displays", during which males and females often cross their bill and press their breasts against each other, while doing soft twittering calls. Pictures from Wolfgang Forstmeier.

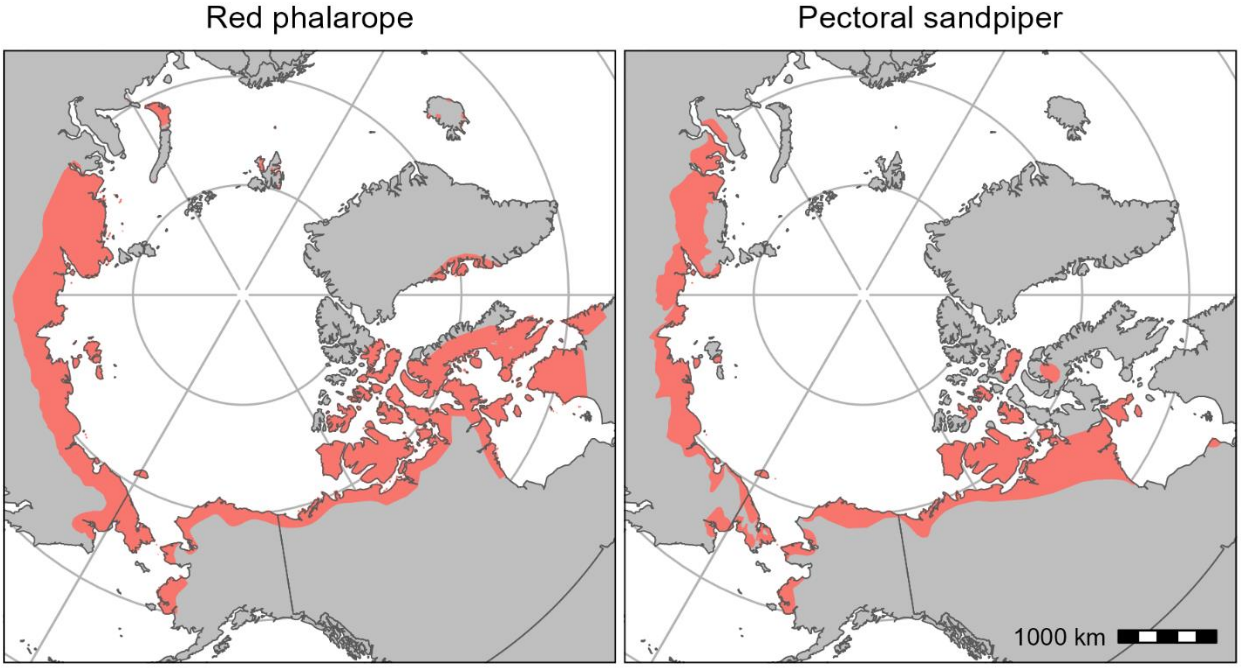

Figure 2. Breeding range of red phalaropes (left) and pectoral sandpiper (right). The breeding range of both species overlaps in most areas, but red phalaropes breed circumpolar in the Northern Hemisphere, while pectoral sandpipers' breeding range is limited to the Arctic coastal plains of east and central Siberia, Alaska and north-west Canada. Breeding range data from BirdLife International.

While sharing the same breeding habitat, the mating system of red phalaropes and pectoral sandpipers is distinctly different. Red phalaropes have typical sex-roles reversed, with females competing more intensely for males and males providing all parental care. Red phalaropes are socially polyandrous, meaning that females can pair with multiple males within one breeding season, and they do this in a sequential fashion (i.e. sequential polyandry). Polyandry is possible in the short Arctic summer, because males and females form a short-term pair bond which can already terminate while egg-laying (typically a 4-egg clutch), and because uniparental care by the males allows females to quickly move on and search another partner. Therefore, this mating system is sometimes also referred to as female-access polyandry, because the local accessibility of available males willing to take care of a clutch plays an important role in the possibility for females to become polyandrous. Despite being a classical example of polyandrous species, only two studies (to my record) are published on the rates of polyandry in red phalaropes. The first conclusive evidence on polyandry came from Schamel and Tracy (1977), which found that 4 of 9 breeding females (44%) laid a clutch for a second social mate. This was followed by Whitfield (1995) who found that 3 of 6 females were socially polyandrous (50%). The only genetic parentage study on red phalaropes detected extra-pair paternity in 6 of 18 (33%) clutches, with clutches containing extra-pair young laid significantly later in the season, which hinted to possible fertilizations with sperm stored from the first mates (Dale et al., 1999). Long sperm, compared to other shorebirds, may suggest intense sperm competition (Johnson and Briskie, 1999). Red phalaropes might arrive paired on the breeding grounds or pair soon after arrival (Mayfield, 1979; Schamel and Tracy, 1977). Pair formation can be initiated by both sexes and is typically driven by sex-biases in the operational sex-ratio, with the sex that is in excess more likely to initiate pair formation (Tracy and Schamel, 1988). Red phalaropes are non-territorial, which allows them to forage benefiting from ephemeral food peaks associated with snow melt patterns (Tracy et al., 2020 and own observations). When paired, they primarily move around together and defend a small area around themselves (Tracy et al., 2020).

In contrast to the polyandrous red phalarope, pectoral sandpipers have a polygynous mating system (Farmer et al., 2020). Male pectoral sandpipers establish display territories of varying sizes, considerably exceeding those observed in traditional lekking species (Lesku et al., 2012; Pitelka, 1959). These territories typically cover most of the suitable breeding area and are strongly guarded against male intruders by doing fight displays, vocalizations ("hooting") and patrolling flights (Pitelka, 1959; Riede et al., 2015). Their main purpose is to defend an area in which they have access to fertile females and possibly to attract females. Females can freely move between male territories for foraging, mate searching and nest site selection (Kistchinskii, 1974; Pitelka, 1959). Females and males only closely associate for courtship and copulation, without the formation of a pair bond, and females provide all the parental care (Farmer et al., 2020; Pitelka, 1959). Male-male competition for mating opportunities is fierce, and under 24 h daylight in the Arctic summer, males significantly decrease their sleep duration to continuously engage in competitive displays, physical fights and court females (Lesku et al., 2012). Most males are not restricted to one territory within the breeding season, but change territories dynamically over multiple potential breeding sites (up to 24 such sites were observed), which in some instances spread throughout the entire breeding range (Kempenaers and Valcu, 2017). Local tenure, defined as the number of days a male stays at one site, depends on the number of available females and predicts local siring success (Kempenaers and Valcu, 2017). Single males were documented to sire up to 22 young locally, but the average male only sired 1.1 young in one location (n = 451) (Kempenaers and Valcu, 2017). Note that many males only stay for one day at one site. Yet, estimating the mating skew for this species is not trivial since it would require the knowledge of the siring success for each breeding site. Most clutches are sired by one father, but 16% of clutches with fully assigned paternity (n = 170) contained multiple (in all cases two) fathers (Kempenaers and Valcu, 2017). Sleep reduction was also directly correlated with siring success, with males that sleep the least being able to sire the highest number of young (Lesku et al., 2012).

Study site and general procedures

We studied red phalaropes and pectoral sandpipers in Utqiaġvik (formerly Barrow), at the northernmost tip of Alaska. The Arctic tundra is a food rich ecosystem, with a short summer in which the sun never sets, which creates a short and intense breeding season for shorebirds (Meltofte, 2017; Steiger et al., 2013). It facilitates polygynous, monogamous and polyandrous species breeding sympatrically in good numbers for scientific studies. Most bird species are not afraid of humans due to their little interaction with them, as a result of the sparsely populated wilderness that distinguishes the Arctic environment. This factor, together with the ideal open structure of the tundra habitat, due to its mainly flat ladscape and treeless vegetation, makes Arctic birds an ideal target for behavioural observations. One problem is that most of the Arctic is not easily accessible, which can complicate the logistics. Utqiaġvik has the benefit of having a commercial airport and a local Iñupiat cooperation (UIC Science) that provides housing, lab space and other logistic support for scientists, which made it with time a science hub for Arctic research and an ideal place for us to study shorebirds with different mating systems.

Our study site was made of 2.5 km^2 of open wet tundra habitat southeast of the former Naval Arctic Research Laboratory (NARL) and Barrow Arctic Research Center (BARC), which are run by UIC Science (Figure 3; 71°19’N 156°39’W). For this thesis, we organized fieldwork for three summer seasons from late May to late July in 2017–2019 and we focussed on red phalaropes. We caught birds as soon as they arrived in the study site using an active approach, where two people handheld mist nets (dimensions: 1.2x12 m or 1.2x24 m) spanned between two poles. Each captured individual was equipped with a U.S. Geological Survey metal band and a distinctive combination of four colour bands, which enabled us to accurately identify and record the behaviour of each individual. Standard morphometric measurements (tarsus, culmen, total head, wing and weight), pictures (head, wing, belly and tail) and blood samples (for DNA extraction and testosterone analysis) were taken from all individuals, as well as cloacal swabs from a subset in 2018 and 2019. In order to follow individuals over a larger distance, we attached 2 g solar-powered Argos Platform Transmitter Terminal (PTT) tags (Microwave Telemetry, Inc.) on 40 females in 2017 and 31 females in 2018. Detailed fine scale movements were collected using 3.5 g solar-powered NanoRadioTag-3 (Milsar Inc.) telemetry loggers on 101 individuals (53 males and 48 females) in 2018 and on 202 individuals (105 males and 97 females) in 2019 (Chapter 2). Each individual was sexed based on plumage characteristics in the field (Tracy et al., 2020). The assigned sex was later confirmed with molecular methods (Chapter 1).

Figure 3. A map with the location of our study site (top, study site indicated in red) and an aerial picture of the north eastern part of the study site (bottom). Our study site is located southeast of the former Naval Arctic Research Laboratory (NARL) and Barrow Arctic Research Centre (BARC) and northwest of the town of Utqiaġvik, at the northernmost tip of Alaska. The study site is primarily made of open wet tundra habitat characterized by high-centred polygons, scattered small ponds and partly wet areas. Picture from Johannes Krietsch (June 2019).

We usually started observing and catching the first red phalaropes that arrived on the first snow free patches close to roads around Utqiaġvik. Once the first red phalaropes arrived on our study site, we systematically monitored the study plot with a team of 2–10 people to conduct *ad lib* observations and to search for nests. Once an individual bird or group of birds was identified, the observer recorded its location with a handheld GPS and described its behaviour (foraging, aggression, display or copulation). Whenever individuals engaged in conspecific interactions such as courtship, contact calls, or aggression, or otherwise foraged within proximity of roughly 5 meters from one another, we documented their presence as being together by recording a single GPS point and the corresponding time. Social pairs commonly remained in close proximity, typically within a few meters of each other, engaging in communication through contact calls, courtship displays, and engaging in copulations. Nests were found by: (a) following males or pairs that showed scraping behaviour (nest preparation) or were observed sitting in scrapes, which is typically indicative of egg-laying preparations and occurs a few days prior to the first egg being laid; (b) following females until they arrived at a scrape or nest to lay an egg; (c) following males that were either flushed off a nest, (accidentally or during rope-dragging, which is systematic search with a rope dragged by two people), or that were taking a break in incubation, until they returned to their nest; (d) inspecting potential nest locations based on the GPS data of males equipped with a GPS tag. Once a nest was found, it was equipped with a temperature sensor (MSR Electronics GmbH) for accurate nest fate determination and to collect detailed incubation and nest fate data.

In order to prevent data loss due to predation, we carefully collected all eggs and exchanged them with artificial plastic eggs designed to closely resemble the real ones in terms of size, weight, and colour. The real eggs were then incubated in a 1502 Digital Sportsman Cabinet Incubator (GQF Manufacturing) under a constant temperature of 38°C and 55% humidity. Once the chicks were close to hatching (i.e. had cracks in the shell due to chicks using their egg tooth to penetrate the shell), the eggs were moved to a 1550 Digital Hatcher (GQF Manufacturing) with the same temperature, but higher humidity (around 70%). The chicks were processed within 10 hours after hatching, by measuring their tarsus and culmen, banding them with a U.S. Geological Survey metal band, and collecting approximately 5 µl of blood from the jugular vein using a 30 gauge, ½” long needle attached to a 0.5 ml syringe (Lanctot, 1994). Subsequently, the chicks were returned to the males and swapped back for the dummy eggs. This procedure enabled us to determine the parentage of nearly all eggs laid within the study site, as well as some eggs from the surrounding area, using a comprehensive analysis of 30 microsatellite markers (Chapter 1).

For the parentage analysis of Chapter 1, we supplemented the field data from our study plot with three other sources, to get a larger sample size: First, we used clutches that our team found outside the study plot and for which we collected blood samples of the chicks. Second, we collaborated with Richard B. Lanctot and Sarah Saalfeld, who collected blood samples from fathers and their offspring as part of a long-term monitoring study in 2003-2006, 2014 and 2017-2019 on nine 0.36 km^2 plots (Saalfeld and Lanctot, 2015; Saalfeld et al., 2019). Third, we got samples from fathers and their offspring from clutches that were removed as part of a study to investigate re-nesting rates of males in 2018 (Cosgrove et al., 2020).

In 2018 and 2019, as part of the GPS tracking of red phalaropes (Chapter 2), we sporadically monitored the larger Utqiaġvik area using all available roads, with the aim to download data from individuals tagged with GPS telemetry loggers that left the study plot. For this, we drove along the roads with a directional antenna (~1500 m downloading range) mounted on a van on a 3 m high pole, or flew a drone (Dji Mavic 2 Pro) at typically 120 m height with an omnidirectional antenna (~1000 m range) attached to it, arriving at times at 2 km from the road network.

For Chapter 3, we used a subset of movement data gathered with 5 g solar-powered Argos PTT tags from 80 pectoral sandpipers that were collected in 2012 and 2014 as part of a detailed breeding study on this species in the same study site (Kempenaers and Valcu, 2017). I was not involved in this field work, but joined a short trip to Utqiaġvik in 2016 to equip males with PTT tags and joined a full breeding season fieldwork on pectoral sandpipers during peak time in 2022.

Aims and thesis outline

The aim of this dissertation is to refine our knowledge of the mating behaviours of two polygamous shorebird species, the polyandrous red phalarope and the polygynous pectoral sandpiper. In particular, we aimed to characterize the social and genetic mating system of the red phalarope, by quantifying the number of social and genetic mates and documenting the copulation behaviour (Chapter 1), and by describing variation in mate guarding intensity in relation to breeding phenology, proxies of mate availability, and other mutually exclusive behaviours, like incubation or mate searching (Chapter 2). Furthermore, we used the large-scale movements of pectoral sandpipers, to understand if the apparently nomadic movements between potential breeding sites (Kempenaers and Valcu, 2017) are influenced by the prevailing wind conditions. This allowed us to speculate whether mate searching behaviour is also influenced by those environmental conditions that influence the cost of movement (Chapter 3). By focussing on two polygamous species with strong intrasexual competition in males or females, I hope to shed light on the selective drivers that lead the evolution of sex-roles, mate searching and mate choice strategies. By doing so, I hope to add general knowledge to our understanding of sexual selection and the evolution of different mating system.

In Chapter 1, we present data that helps characterize the social and genetic mating system of red phalaropes, namely the number of polyandrous females and re-nesting males and females; the frequencies of extra-pair paternity, and the occurrence of within- and extra-pair. Furthermore, we use this comprehensive data set to test the long standing “sperm-storage” hypothesis (Oring et al., 1992) in sequentially polyandrous species. This hypothesis suggests that extra-pair paternity in these species can be explained by sperm stored by females from within-pair copulations with a previous mate which is then used to fertilize eggs in a subsequent clutch that is cared for by another male. We test multiple predictions of this hypothesis and evaluated other explanations for the occurrence of extra-pair paternity in this species, such as extra-pair copulations and rapid switching between potential mates.

In Chapter 2, we investigated whether mate guarding behaviour, which is typically considered to be a male strategy to protect paternity, can be mutually beneficial for males and females in sex-role reversed species like the red phalarope. Therefore, we examined the variation in time spent together and biases in separation movements in relation to the female’s fertile period, based on clutch initiation, and in relation to season, as a proxy for mate availability (i.e. operational sex ratio). Furthermore, we studied whether the investment in mate guarding has a trades-off with parental care, for the males, or with subsequent mate searching, for the females. Ultimately, we tested whether variation in mate guarding intensity was linked to the occurrence of extra-pair paternity.

In Chapter 3, we explored whether environmental conditions, in particular the prevailing wind conditions, can influence the breeding site sampling and therefore mate searching behaviour of pectoral sandpipers. Since the mating opportunities in distant sites are unpredictable and time is an important resource in the short Arctic breeding season, males’ apparently nomadic movements could be influenced by the characteristics of the medium they move in. Therefore, we first described the wind conditions experienced by males and we analysed the effect of wind on ground and air speed, and subsequently tested the hypothesis that individuals decided on which part of the breeding range to move based on the prevailing wind conditions.

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Chapter 1

Extrapair paternity in a sequentially polyandrous shorebird: limited evidence for the sperm storage hypothesis

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In socially monogamous species, extrapair paternity typically results from extrapair copulations, but it can also be due to rapid mate switching. Oring, Fleischer, Reed, and Marsden (1992, *Nature, 359* (6396), 631–633) proposed a mechanism to explain the occurrence of extrapair paternity in sequentially polyandrous species: sperm stored by females from within-pair copulations with a previous mate could fertilize eggs in the clutch of a subsequent male. Despite being proposed decades ago, evidence for this hypothesis remains limited. We studied social polyandry, extrapair paternity and copulation behaviour in a population of the red phalarope, *Phalaropus fulicarius*, a nonterritorial, sex role-reversed shorebird, with male-only care, in Utqiaġvik, Alaska. We tested multiple predictions from the 'sperm storage' hypothesis. Extrapair paternity occurred in 11% (37/334) of the nests and 4% (42/1182) of the eggs were sired by a male other than the incubating parent. Although a female's initial mate occasionally sired offspring in her next clutch, our results suggest that sperm stored from a previous mate does not play a major role in explaining the occurrence of extrapair paternity in this sequentially polyandrous species. Instead, extrapair paternity was generally due to extrapair copulations by both sexes during the period between pair establishment and early incubation and to rapid mate switching by females in the context of attempts to acquire multiple care-giving males.

Classical polyandry, where one male breeds exclusively with one female, while individual females may socially pair with multiple males, is a rare mating system. In birds, it has been described for less than 1% of all species (Cockburn, 2006; Oring, 1986). In this system, typical sex roles are reversed: males provide all parental care, while females compete for resources or mates. Two types of classical polyandry can be distinguished: simultaneous and sequential (Oring, 1986). In simultaneous polyandry, females defend multipurpose territories and, depending on the quality of the territory, can attract and pair with multiple males simultaneously (Chen et al., 2008; Emlen et al., 1998; Goymann et al., 2004). In sequential polyandry, females form a pair bond with one male at a time but can rapidly switch to a new mate after having laid a full clutch. Both sexes are typically nonterritorial and females do not defend resources, but rather compete for access to males (Colwell & Oring, 1988; Kålås & Byrkjedal, 1984; Oring et al., 1992; Reynolds, 1987; Schamel & Tracy, 1977). A common characteristic of socially polyandrous species is that the reproductive rate of females is limited by access to males, which makes these species particularly interesting for studies of sexual conflict (Owens, 2002).

In classical polyandrous species, females can increase their reproductive success by mating with multiple males within one breeding season and producing a clutch for each of them. Male reproductive success appears to be more constrained, as males can typically only raise one brood per season. However, males can increase their reproductive success by siring extrapair offspring. Because paternity loss is costly, males have evolved strategies to avoid raising offspring sired by other males, such as intense mate guarding and frequent copulation (Emlen et al., 1998; Schamel, Westneat et al., 2004). Indeed, it has been argued that the evolution of male-only care is linked to high certainty of paternity (Sheldon, 2002; Trivers, 1972; Wright, 1998). Thus, the observation that polyandrous species often show similar or even higher

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rates of extrapair paternity than closely related monogamous species (e.g. in coucals: Muck et al., 2009; Safari & Goymann, 2018; in shorebirds: see Appendix Table A1) seems puzzling at first.

To understand the causes of paternity loss in polyandrous species, it is critical to evaluate the mechanism of loss in simultaneous and sequential polyandry separately. In simultaneously polyandrous species, males typically lose paternity to co-mates of the female, because females copulate with multiple 'harem' males while producing eggs for one of them (Emlen et al., 1998; Safari & Goymann, 2018). In contrast, in sequentially polyandrous species, females pair with one male at a time, and paternity loss is thought to result exclusively from stored sperm from a previous partner that fertilizes one or more eggs in a subsequent clutch (Dale et al., 1999; Oring et al., 1992; Schamel, Westneat et al., 2004). This mechanism, in which extrapair fertilization is the result of within-pair copulations with the previous partner, is referred to as the 'sperm storage hypothesis'. Thus, in polyandrous species, early breeding males might increase their reproductive success by pursuing frequent within-pair copulations to ensure that their mate's sperm storage tubules (Birkhead & Møller, 1993; Frey & Goymann, 2009; Holt & Fazeli, 2016) are filled with sperm, which should increase the probability that they fertilize eggs in their mate's subsequent clutch. Females, in turn, might also benefit from a high within-pair copulation rate if storing sperm from early breeding males yields offspring of higher genetic quality in subsequent clutches (Dale et al., 1999; Oring et al., 1992).

Despite being proposed 30 years ago, evidence for the sperm storage hypothesis as an explanation for the occurrence of extrapair paternity in sequentially polyandrous birds remains limited and indirect. The hypothesis that males could lose paternity due to stored sperm from the previous partner was first suggested by Whitfield (1990), who observed that male red-necked phalaropes, *Phalaropus lobatus*, avoided pairing with females that had previously laid a clutch (although it remains unclear how males can identify such females). The key prediction of the hypothesis is that extrapair offspring are sired by a female's previous social mate. However, no study has shown that this is indeed the case. Oring et al. (1992) reported the occurrence of extrapair offspring in seven of 34 clutches of sequentially polyandrous spotted sandpipers, *Actitis macularius*. Because no extrapair copulations had been observed, the authors suggested that a previous mate had sired these offspring with sperm stored by females (for 3–31 days), but the identity of the sires could not be determined with certainty (Oring et al., 1992). The sperm storage hypothesis further predicts that extrapair offspring should not occur in the first clutch of a female, but only in subsequent clutches laid for a different male. In support of this prediction, Schamel, Westneat et al. (2004) detected extrapair offspring in three of six known second clutches from polyandrous female red-necked phalaropes, compared to none in 25 presumably first-laid clutches and one in 16 monogamous replacement clutches. However, the identity of the sires of the extrapair offspring was not determined (Schamel, Westneat et al., 2004). In another study on red phalaropes, *Phalaropus fulicarius*, Dale et al. (1999) found extrapair offspring in six of 12 late-laid clutches, but in none of six early clutches. Although the authors did not have information about the females that produced the clutch or about clutch order, they assumed that clutches produced later in the season were more likely to be second (or subsequent) clutches of polyandrous females. Under this assumption, the sperm storage hypothesis predicts that extrapair paternity should only occur in late-laid clutches, which is what they found. Similarly, Owens et al. (1995) found that the only two extrapair young detected in the Eurasian dotterel, *Charadrius morinellus*, were from two late-laid clutches (out of 22).

Here, we report on a study of extrapair paternity in a population of red phalaropes breeding in the high Arctic. We combined data from a 17-year nest-monitoring study near Utqiaġvik, Alaska, where DNA from offspring and incubating males was occasionally collected, with data from an intensive 3-year study focused on a ca. 2.5 km^2 study area, where we caught and marked almost all adults, conducted behavioural observations on males and females, monitored nesting attempts of individual females, and obtained DNA from almost all adults and offspring.

The main aim of our study was to test the sperm storage hypothesis as an explanation for extrapair paternity in the red phalarope. Specifically, we tested the following predictions from this hypothesis (Dale et al., 1999; Oring et al., 1992; Schamel, Westneat et al., 2004). (1) Extrapair paternity should be more common in years with high rates of social polyandry because females producing clutches for more than one male could use stored sperm from a previous mate. (2) Extrapair paternity should be more common in second-laid clutches of socially polyandrous females compared to first-laid clutches and replacement clutches produced by a female with the same mate (after failure of the previous clutch, e.g. due to predation). (3) Extrapair paternity should mainly occur in clutches laid later in the season, assuming that a larger proportion of these clutches are second- or third-laid clutches of polyandrous females. This assumption is important, because females are not territorial and can move over large distances (Krietsch, Valcu et al., 2021), making it difficult to determine whether a later-laying female has already produced a clutch with another male. (4) Extrapair offspring in a second clutch of a polyandrous female should be sired by the male that cares for the first clutch. This is the strongest prediction of the sperm storage hypothesis; the first three predictions are also consistent with other hypotheses (e.g. related to the occurrence of extrapair copulations). To test these predictions, we used data from behavioural observations in combination with a molecular parentage analysis to determine all instances of extrapair paternity (genetic polyandry), social polyandry and renesting attempts and to identify the extrapair sires.

As a secondary aim, we considered and evaluated other explanations for the occurrence of extrapair paternity than sperm stored from the previous social mate, such as extrapair copulations and rapid switching between potential mates (Fig. 1).

METHODS

Study Species, Study Site and General Procedures

The red phalarope is a migratory shorebird with a pelagic lifestyle during most of the year. It only comes to land to breed in the high Arctic and has a circumpolar distribution (Tracy et al., 2020). Females lay up to three clutches during the short arctic summer (Schamel & Tracy, 1977). Previous studies on this species indicated high rates of sequential polyandry compared to other polyandrous birds, but sample sizes were small (percentage of females that laid clutches for more than one male: 44% (4/9), Schamel & Tracy, 1977; 50% (3/6), Whitfield, 1995). In the only other genetic parentage study on red phalaropes, extrapair paternity was detected in 33% of all clutches (6/18; Dale et al., 1999).

We studied red phalaropes in wet tundra habitat near Utqiaġvik (formerly Barrow), Alaska (71°19′N, 156°39′W) between late May and late July 2003–2019. Most of the data reported here come from an intensive study in 2017–2019 on a 2.5 km^2 plot, where we conducted behavioural observations and sampled DNA of one or both parents and their offspring for 95% of 174 nests (hereafter referred to as the 'intensive study', Fig. 2). We supplemented this information with three other sources of data, which were obtained as part of other studies. First, we obtained DNA samples from 57% of

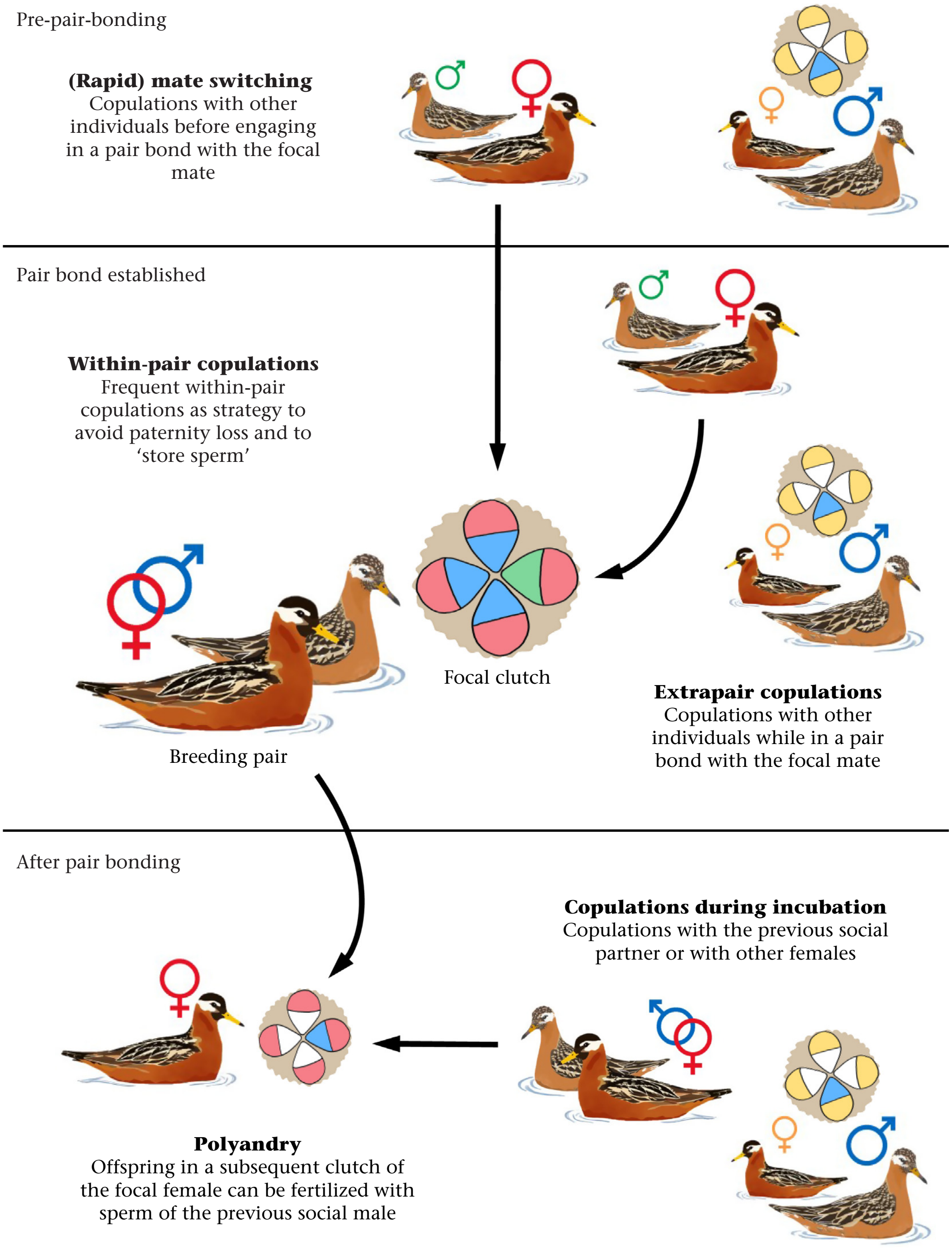

Figure 1. Schematic illustration of behaviours that could lead to extrapair paternity in the sequentially polyandrous red phalarope. The focal breeding pair is shown in red (female) and blue (male). Before establishing a pair bond, the focal female and the focal male might engage in copulations with other males (shown in green), respectively with other females (shown in yellow), as part of courtship rituals. Extrapair paternity could then arise by (rapid) mate switching in the clutch of the focal female and, from the focal male's perspective, in a clutch of another female (yellow). Copulations with other individuals (indicated in green and yellow) can also lead to extrapair paternity while the pair bond is established (i.e. through extrapair copulations). Frequent within-pair copulations can be a strategy of the focal male to avoid paternity loss but can also result in extrapair young in the subsequent clutch of the focal female. This mechanism is linked to the focal female becoming socially polyandrous and is referred to as the 'sperm storage hypothesis'. After the pair bond with the focal female is terminated (clutch completed), the focal male can still sire extrapair offspring by copulating with the focal female (if she produces a subsequent clutch for another male) or with other fertile females. The colours in the egg represent the genetic parents (white indicates the social mate of a female, except in the focal clutch, where the sire is the focal male indicated in blue). The figure shows both how the focal male can lose paternity (to the male indicated in green; central part of the figure) and how the focal male can obtain extrapair offspring (with his previous mate, indicated in red, or with another female, indicated in yellow).

30 clutches found outside the 2.5 km^2 study plot ('outside plot') whose fathers were initially captured within the intensive study plot. Second, we collected DNA samples from social fathers and their offspring from 15% of 849 nests found on nine 0.36 km^2 plots as part of a long-term monitoring study (data from 2003–2006, 2014 and 2017–2019; described in Saalfeld & Lanctot, 2015; Saalfeld et al., 2019; 'long-term monitoring', Fig. 2). Third, we obtained DNA samples from all social fathers and their offspring from

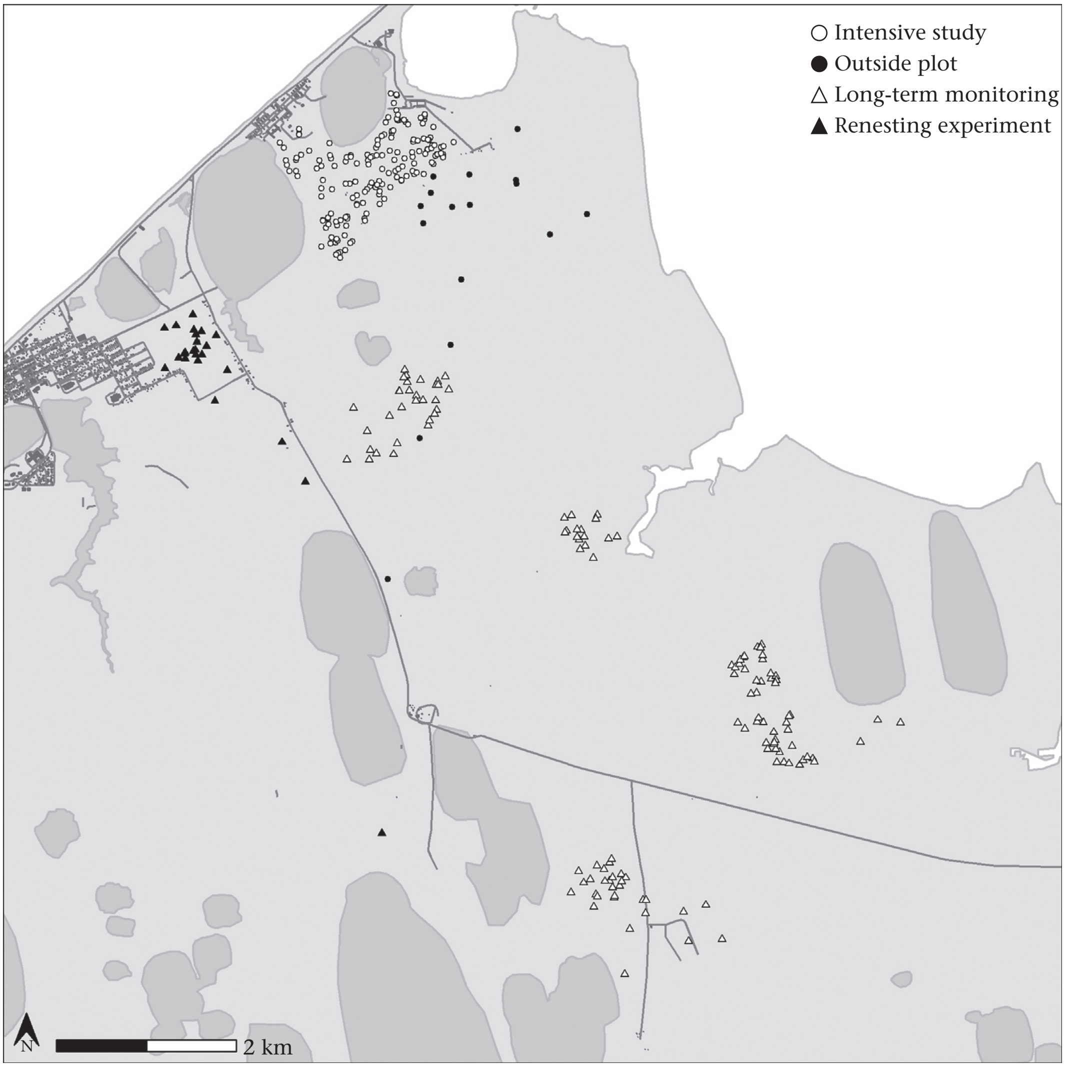

Figure 2. Location of sampled red phalarope nests near the village of Utqiaġvik, Alaska (71°19′N, 156°39′W). Nests belonged to four categories (see Methods). (1) 'Intensive study': nests found within our intensive study plot (2017–2019, *N* = 165); (2) 'outside plot': nests of birds banded in our intensive study plot but found breeding outside it (2017–2019, *N* = 17); (3) 'long-term monitoring': nests sampled during a long-term monitoring study (2003–2019, *N* = 128); (4) 'renesting experiment': nests collected as part of a study to investigate renesting rates of males (2018, *N* = 24). Tundra habitat is indicated in light grey, water bodies in darker grey and the sea in white. Dark grey lines represent roads. Map projection: polar Lambert azimuthal equal area with longitude origin in Utqiaġvik. Map data from OpenStreetMap.

24 clutches that were removed as part of another study to investigate renesting rates of males in 2018 (see Cosgrove et al., 2020 'renesting experiment', Fig. 2).

Field Procedures

To provide the most comprehensive test of the different predictions of the sperm storage hypothesis, we used data from four sources that differed in the way they were collected. In the intensive study site, we focused on obtaining the most complete parentage data (by sampling most males and females) in combination with behavioural observations. Data from the other three sources were collected more opportunistically. All data sources were used to estimate the frequency of extrapair paternity and to evaluate the effect of breeding phenology. The data from the intensive study site were used to assess the rate of polyandry and renesting, determine clutch order, evaluate the characteristics of extrapair sires and analyse behavioural interactions. We describe each source separately and explain the differences in research methodology (e.g. capture techniques, monitoring frequency).

Intensive study

In 2017–2019, we observed the first red phalaropes within the 2.5 km^2 study plot between 1 and 6 June, 5–9 days after red phalaropes had first been observed on snow-free tundra patches along the streets of Utqiaġvik. We caught birds with handheld mist nets as soon as they arrived in the study site (total number of individuals: 138 in 2017, 203 in 2018 and 319 in 2019). Each captured individual was sexed based on plumage characteristics (Tracy et al., 2020), which was later confirmed with molecular methods (see below), and banded with a U.S. Geological Survey metal band and a unique combination of four colour bands that allowed us to identify and document the behaviour of individual birds. We took a 5–10 μl blood sample by puncturing the brachial vein and stored the blood in 1 ml Queen's lysis buffer (Seutin et al., 1991) at room temperature for later analysis.

After birds arrived, we searched for nests daily across the study site with a team of 2–10 people by (1) following males or pairs that made scrapes or sat in scrapes, behaviour typically shown a few days before the first egg was laid, (2) following females until they went to a scrape or nest to lay an egg, and (3) following males after flushing them off a nest (accidently or by rope dragging) or during a natural incubation break until they returned to their nest. Most nests were found during laying (78% of 174). Whenever a nest was found, we either identified the attending male based on its colour-band combination, or, if the male was still unbanded (13% of 174 nests), we trapped it with a handheld mist net near the nest and processed it as described above. Then, to avoid loss of DNA samples from offspring should nests be depredated, we collected all eggs and replaced them with dummy eggs that resembled real eggs in size, weight and colour. We marked each collected egg with the nest identity and placed it in an incubator (GQF Manufacturing Company Inc., Savannah, GA, U.S.A., Sportsman 1502). During the incubation period, we candled eggs to monitor their development. Once eggs showed signs of pipping, we moved them to a hatcher (GQF Manufacturing Company Inc., Hatcher 1550). We allowed each chick to dry after it hatched, before banding it and taking a small blood sample (3–5 μl) from the jugular vein using a G30x0.5'' (0.3x13 mm) sterile needle on a syringe (after Hoysak & Weatherhead, 1991) and stored it in Queen's lysis buffer (see above). The chicks were then returned to the nest of an incubating male (see Ethical Note). We collected a tissue sample from the dead embryos of all eggs that did not hatch (65 eggs from 47 clutches, 11% of all eggs) or from the embryos that had to be euthanized, because no more males were available to care for the chicks (due to predation of nests with dummy eggs; 156 eggs from 50 clutches, 26% of all eggs). Samples were placed in a screw-cap tube with 1 ml RNAlater (Sigma-Aldrich) for later parentage analysis.

We determined nest initiation dates (i.e. date the first egg was laid) either by (1) subtracting 1 day for each egg in the nest for clutches found during egg laying (assuming one egg per day was laid, 49% of nests), (2) subtracting 1 day for each egg in the clutch plus the mean incubation period of 17 days (SD = 0.8, range 15–20 days) for eggs hatching in the incubator (based on 58 nests with known laying date) or 19 days for naturally incubated clutches (35% of nests; Weiser et al., 2018), or (3) subtracting the estimated developmental age based on flotation (Liebezeit et al., 2007) and clutch size for unsuccessful nests (depredated or deserted, 16% of nests). When females were known to lay multiple clutches (based on parentage analysis), we categorized each clutch as initial, second or third based on laying date. Although previous clutches may have been missed (e.g. because a female first laid outside the study area or laid eggs in a nest that was quickly depredated), we classified the remaining females as 'single-clutch females' (i.e. with only one known clutch).

The 2–10 people that systematically monitored the study plot also conducted ad libitum observations. Once a marked individual was identified, the observer recorded its location with a handheld GPS and described its behaviour (foraging, aggression, display or copulation). If individuals interacted with conspecifics (courtship, contact calls or aggression), or foraged within ca. 5 m from each other, we recorded them as being together (i.e. one GPS point and time). Social pairs typically stayed within a few metres of each other, communicated by uttering contact calls, and engaged in courtship behaviour and copulations. We defined breeding pairs a posteriori as a male and female that subsequently were determined to have a clutch together. We assessed this based on direct observations (i.e. females seen on or close to the nest with the male during scraping or egg laying) and on parentage analysis. We classified all interactions and copulations between members of a breeding pair as 'within-pair' and those between other opposite-sex individuals as 'extrapair'. Note that interactions between the breeding male of the first clutch of a polyandrous female are defined as within-pair interactions when referring to the first clutch, but as 'extrapair' interactions from the perspective of the second clutch (incubated by a different male).

Other data sources

'Outside plot' nests were those belonging to males caught and banded within the intensive study plot, but physically located outside the intensive study plot ($N = 17$). These nests were found either accidently by flushing a male off the nest or by tracking GPS-tagged males (Krietsch, Valcu et al., 2021). All other procedures were identical to those of the intensive study. The 'long-term monitoring' data come from four (2003) to six (all other years) 0.36 km^2 plots (with a total of nine different plots across the study period). Nests were located using the same methods as described for the intensive study, but with observers searching nests of all shorebird species. DNA samples from the incubating male and its offspring were collected from 128 nests in 2003–2006, 2014 and 2017–2019 (described in Saalfeld & Lanctot, 2015; Saalfeld et al., 2019). Incubating males were caught on their nest using a modified bow-net trap and banded and sampled for blood as described above. Nests were visited around the time of hatching and a blood sample was taken from newly hatched offspring, while embryo tissue was sampled from unhatched eggs. In the 'renesting experiment' study, 24 clutches were removed to evaluate the propensity of males to renest (Cosgrove et al., 2020). Most males and females were caught before incubation with handheld mist nets; a few males were caught during incubation with a modified bow-net trap. All adults were

Table 1
Frequency of extrapair paternity for each year and data source (see Methods)

Year	Data type	EPY (%)	EPY/total	Nests with EPY (%)	Nests with EPY/total	Males genotyped	Females genotyped
2019	Intensive study	4	14/347	13.5	13/96	149	170
2019	Long-term monitoring	3.5	3/86	11.1	3/27	53	0
2019	Outside plot	4.1	2/49	14.3	2/14	15	31
2018	Intensive study	1.5	2/134	5.7	2/35	95	108
2018	Long-term monitoring	4	2/50	12.5	2/16	48	0
2018	Outside plot	0	0/8	0	0/2	44	78
2018	Renesting experiment	2.4	2/85	8.3	2/24	33	20
2017	Intensive study	0.8	1/119	2.9	1/34	65	72
2017	Long-term monitoring	5	2/40	16.7	2/12	12	0
2017	Outside plot	0	0/3	0	0/1	51	59
2014	Long-term monitoring	10	7/70	20	4/20	20	0
2006	Long-term monitoring	4.1	2/49	14.3	2/14	13	0
2005	Long-term monitoring	7.3	4/55	18.8	3/16	17	0
2004	Long-term monitoring	2.6	1/38	10	1/10	12	0
2003	Long-term monitoring	0	0/49	0	0/13	13	0
Total		3.6	42/1182	11.1	37/334	638	536

The total percentage of extrapair young (EPY), the percentage of nests with EPY and the total number of candidate parents genotyped are shown. The mean clutch size of all sampled nests was 3.7 (SD = 0.6; range 1–4 eggs, $N = 334$).

blood sampled. We placed all clutches in the incubator for ca. 5 days, euthanized them and sampled tissue from each embryo.

Ethical Note

All procedures were approved by the US Geological Survey Bird Banding Laboratory (permit numbers 23520 and 23269), the Alaska Department of Fish and Game (permit numbers 17–149, 18–146 and 19–143), the US Fish and Wildlife Service (permit number MB210494-0), the animal care and use committee (through the U.S. Fish and Wildlife Service 2009–011, 2012–006, 2015–005 and 2018–005) and the North Slope Borough and the Ukpeaġvik Iñupiat Corporation.

After hatching in the incubator, offspring were left to dry and brought to the nest of an incubating male (the social male,

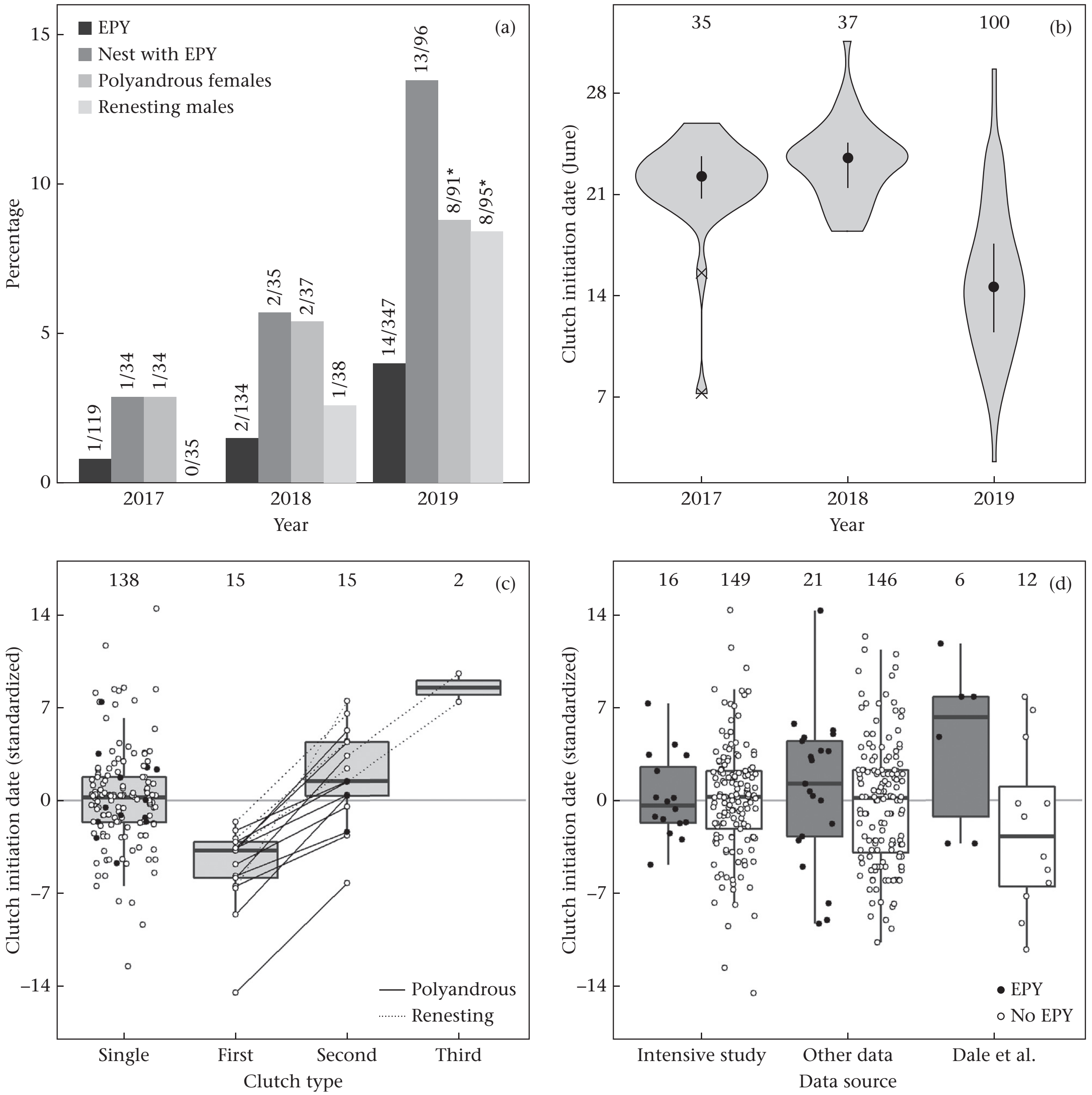

Figure 3. Frequency of extrapair paternity, social polyandry and renesting attempts and the link between extrapair paternity and clutch initiation dates. Numbers at the top indicate sample sizes (number of eggs, nests or unique females and males). (a) The total percentage of extrapair young (EPY), the percentage of clutches with extrapair young, the percentage of females that were socially polyandrous and the percentage of males that renested after clutch failure for each year (data from the intensive study). In 2019, two polyandrous females laid their first clutch in the intensive study plot and their second clutch outside this area, while three males renested outside the intensive study plot (indicated by *). When these cases are excluded (i.e. only nests within the intensive study plot considered), 6.6% of females were socially polyandrous and 5.3% of males renested in that year. (b) Clutch initiation date (i.e. date on which the first egg was laid; data from the intensive study plot). For each year, we show the median (dot), 25th–75th percentile (lines), outliers (x) and a violin plot illustrating kernel probability densities, i.e. the width of the grey area represents the proportion of data located there (maximum width: 7 nests in 2017, 11 nests in 2018 and 10 nests in 2019). (c) Clutch initiation date (standardized by subtracting the mean of each year) for different types of clutches (the single known clutch of a female; the first, second and third clutch of a polyandrous or a renesting female). Sequential clutches were either laid for a different male (polyandrous female, $N = 11$, solid lines) or for the same male (renesting, $N = 6$, dotted lines). Open and filled circles indicate nests without and with extrapair young, respectively. (d) Clutch initiation dates for nests with extrapair young (grey bars) and without extrapair paternity (white bars), shown separately for the intensive study plot, all other data sources and the data from Dale et al. (1999). Box plots show the median (central line), 25th–75th percentile (limits), and minimum and maximum values without outliers (whiskers).

whenever possible) after up to ca. 12 h in groups of four to five. All males readily accepted and brooded the chicks. When no more males were available to care for offspring (due to predation of nests with dummy eggs) we euthanized eggs by freezing them at -40 °C, consistent with the recommendations of the American Veterinary Medical Association (Leary et al., 2020).

Parentage Analysis and Molecular Sexing

The mean clutch size of all sampled nests was 3.7 (SD = 0.6; range 1–4 eggs, *N* = 334). We sampled all offspring in 86% (286/334) of clutches in total and in 98% (161/165) of the clutches in the intensive study. DNA was extracted from blood using the NucleoSpin Blood Quick Pure Kit (Macherey-Nagel GmbH, Düren, Germany), from large tissue samples using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), and from small tissue samples (e.g. germinal disc of unhatched eggs) using a standard phenol–chloroform protocol (Sambrook & Russell, 2001). Of the 165 collected clutches from the intensive study, 20 (12%) included at least one undeveloped egg (i.e. an egg where only the germinal disc was visible); this corresponds to 3% (20/602) of all eggs. We extracted DNA from 90% (18/20) of these undeveloped eggs and amplified both paternal and maternal alleles. Hence, we concluded that these eggs were fertilized and suffered early embryo mortality. The remaining two eggs (0.3%, *N* = 602) could have been infertile.

We genotyped the samples using a set of 30 polymorphic microsatellite markers and one sex chromosome-linked marker (for details see Table A2). Microsatellite amplification was performed in multiplexed PCRs using the Qiagen Type-it Microsatellite PCR Kit (Qiagen) with four primer mixes containing five to nine primer pairs. Each 10 μl multiplex PCR contained 20–80 ng DNA, 5 μl of the 2x Type-it Microsatellite PCR Master Mix and 1 μl of one of the four primer mixes. For fragment length analysis, an aliquot of 1.5 μl of each PCR product was added to 13 μl formamide containing the GeneScan 500 LIZ Size Standard, then heat denatured and resolved in POP7 polymer on an ABI 3130xl Genetic Analyzer (ThermoFisher Scientific). Raw data were analysed and alleles assigned using the GeneMapper 4.0 software.

We conducted parentage analysis using Cervus 3.0 (Kalinowski et al., 2007). For each year separately, we included all adult males and females observed and captured in the focal year as candidate parents. For the intensive study, we first ran a simulation of maternity with all candidate females, assuming a 1% genotyping error and 90% of the mothers sampled. We then determined the most likely mother using delta LOD scores (i.e. the difference in LOD scores between the most likely and the second most likely candidate female). We then used the following rules to assign maternity: (1) a confidence level of 95%, (2) no more than two loci where the alleles of the candidate female did not match the offspring alleles and (3) in the rare case where two females had 0–1 mismatches with the offspring, we assigned maternity to the one that was the mother of all other offspring in the same clutch. Second, we ran a simulation of paternity with all candidate males, assuming that 95% of the fathers were sampled. We then determined the most likely father by including the assigned mother (if any) as the known parent and using delta LOD score for the parent pair (if the mother was assigned). We only assigned paternity if the candidate male–mother combination was assigned with high confidence (95%) and had no more than one additional (male) mismatch. For those offspring from the intensive study for which one or both genetic parents were not identified, we further included adults sampled or observed in the previous or subsequent year as candidates.

For all nests that did not belong to the intensive study, we evaluated whether the male caught on the nest was the genetic father, based on a positive LOD score and a maximum of two mismatches. When females were sampled as well ('renesting experiment' and 'outside plot'), we determined the most likely mother by including the assigned father as the known parent and used the delta LOD score of the parent pair.

The set of microsatellite markers had the following properties: mean number of alleles = 17, combined nonexclusion probabilities for the first parent = 3.5×10^{-7}, for the second parent = 1.9×10^{-11}, for a parent pair = 5.2×10^{-19}. We assigned the mother to 93% of all clutches (153/165) from the intensive study and to 12% of all other clutches (20/169). We found no cases of intraspecific brood parasitism. We assigned the father to 98% of all offspring from the intensive study (587/600) and to 95% of all offspring from all other clutches (553/582).

Data Analyses

All statistical analyses were performed with R version 4.0.3 (R Core Team, 2020) using RStudio (RStudio Team, 2020). Details on the statistical tests are described in the Results.

RESULTS

Frequency of Extrapair Paternity, Social Polyandry and Renesting

Overall, extrapair paternity occurred in 11% (37/334) of the nests and 4% (42/1182) of all offspring were sired by a male other than the incubating one (Table 1). In the intensive study, the frequency of extrapair paternity was higher in 2019, compared to 2017 and 2018, but the year effect was not significant (generalized linear model with occurrence of extrapair paternity in a brood (yes/no) as the dependent variable and year as explanatory variable: $\chi^2_1 = 4.57$, *P* = 0.10). The overall frequency of extrapair paternity did not differ between nests from the intensive study and those from the three other data sources (generalized linear model with occurrence of extrapair paternity in a brood (yes/no) as the dependent variable and data source as explanatory variable: $\chi^2_1 = 0.63$, *P* = 0.43). In nests with extrapair paternity, 89% (33/37) contained only one extrapair young (out of two to four eggs), three (8%) had two extrapair young (out of four eggs) and one (3%) had three extrapair young (out of four eggs). When multiple extrapair young were present within a single nest, they were sired by a single father, except for one nest with two extrapair young that were sired by different extrapair fathers.

In the intensive study, at least 7% of females (11/162, range 3–9%; Fig. 3a) were socially polyandrous, i.e. they produced

Table 2
Characteristics of red phalarope males that sired extrapair young (EPY) and had their own nest during the same breeding season

Identified sire of EPY	Social female before	Difference in initiation date between own clutch and clutch where EPY sired (days)	Distance between own nest and nest where EPY sired (m)
1	Yes	-3	52
2	Yes	-5	1474
3	No	-10	60
4	No	-1	37
5	No	-6	17
6	No	-4	17
7	No	-2	132
8	No	+1	1037
9	No	+1	246

The difference in the start of laying and the physical distance between the male's own clutch and the clutch in which he sired extrapair offspring are shown. In two cases, the extrapair father was the previous breeding partner of a polyandrous female.

clutches for multiple males within one breeding season of which at least one was in the intensive study plot. Of all males that had a nest in the intensive study plot, at least 5% (9/168, range 0–8%; Fig. 3a) renested after clutch failure; three of these renests were outside the intensive study plot. Of all 11 renesting males (all data sources combined), six renested with the same female, and of those six, two were the third clutch of a polyandrous female. The other five males renested with a new partner.

In the intensive study, rates of extrapair paternity, social polyandry and renesting tended to be higher in 2019 (Fig. 3b). The clutch initiation period, i.e. the difference between the first and last clutch initiation date, was more than three times longer in 2019 than in 2017 (2017: 7 days; 2018: 13 day; 2019: 27 days; excluding outliers; see Fig. 3b).

Extrapair Paternity and Clutch Order

No extrapair young occurred in the 15 first clutches of females known to lay multiple clutches (including four replacement nests with the same male) and in six replacement clutches that did not involve mate change (including the only two known third clutches of individual females in our sample), whereas extrapair paternity did occur in three of 11 second clutches (27%) of socially polyandrous females. Consequently, the percentage of clutches that contained extrapair young tended to be higher after a female changed mates (socially polyandrous females; 27%, $N = 11$) than when no mate change occurred (presumed first clutches and replacement clutches for the same male; 0%, $N = 21$; Fisher's exact test: $P = 0.055$).

However, within the intensive study plot, extrapair paternity also occurred in 10% (14/138) of clutches laid by females that only produced a single clutch and in one of three instances where a different female replaced a lost clutch of a male. Although these are presumably first clutches, we cannot exclude that (some of) these 141 females (138+3) had laid a clutch elsewhere before.

Extrapair Paternity and Breeding Phenology

First, we evaluated whether second or third clutches were indeed laid later in the season (underlying assumption of Dale et al., 1999). Females that laid multiple clutches within the same breeding season ($N = 15$, with a different or the same mate) started their first clutch on average 5.1 days before the mean first egg date of all clutches within the intensive study plot, whereas second clutches were on average initiated 1.7 days after this mean lay date (Fig. 3c). When considering only second clutches of polyandrous females ($N = 11$), their average initiation date was only 0.5 days after the mean first egg date.

Second, we report the time interval between sequential clutches to evaluate how long sperm would have to be stored by females to fertilize eggs in the next clutch. The difference in laying date between the first and second clutch of a polyandrous female was on average only 6.1 days (range 3–9 days, $N = 11$). This means that the second clutch was laid on average only 2.4 days (range 0–5 days, $N = 11$) after the first one was completed. In comparison, females that produced a replacement clutch for the same male started this clutch on average 3.4 days (range 3–5 days, $N = 6$) after the first clutch failed. Two of these replacement clutches were third clutches of females (Fig. 3c). When a different female produced a clutch for a male that lost his first clutch, this clutch was on average initiated 3.5 days (range 2–6 days, $N = 4$) after the first clutch failed.

Contrary to the prediction of the sperm storage hypothesis, nests that contained extrapair young were not initiated later in the season than nests without extrapair young (Fig. 3d; generalized linear model with occurrence of extrapair paternity in a brood (yes/no) as dependent variable and standardized lay date, centred around the annual mean, as explanatory variable; intensive study: effect size = -0.008, SE = 0.06, $Z = -0.14$, $P = 0.89$; other data sources: effect size = -0.04, SE = 0.05, $Z = -0.94$, $P = 0.35$).

Characteristics of the Extrapair Sires

In total, we identified the sire of 26% (11/42) of the extrapair young in 37 clutches. In the intensive study plot, we identified the sire of 47% (8/17) of the extrapair young while for the other data we identified the sire of only 12% (3/25) of these young. Nine of the identified extrapair males cared for their own clutch during the same season, which was initiated on average 3 days before the clutch in which they sired extrapair young (Table 2). The distance between the male's own nest and the nest in which he sired extrapair young varied between 17 m and 1.5 km (median = 60 m, Table 2), while the distance to the nearest nest within the intensive study plot varied between 11 m and 295 m (median = 72 m, SD = 51 m). The other two identified extrapair sires bred within the intensive study plot in the previous year but were not observed in the focal year.

Three second clutches of 11 polyandrous females contained one extrapair young. In two of these cases, the extrapair sire was the previous social mate of the female and the second clutches were laid only 1 and 2 days after completing the respective first clutches. In the third case, the extrapair sire had his own clutch with a different female, which was completed 6 days before the initiation of the clutch in which he sired extrapair young. In the intensive study, only one extrapair young (6% of 17) could be assigned to the previous male of a polyandrous female, whereas for five extrapair young (29% of 17) the assigned extrapair sire was not the previous social male, but a male that tended a clutch laid by a different female. For the majority of extrapair young (11 of 17, 65%), the mechanism that led to extrapair paternity remains unclear; two additional identified sires were males without a known nest (could have bred outside the study site), and the other sires could not be identified.

Timing of Within- and Extrapair Interactions

Based on ad libitum observations in the intensive study plot, within-pair interactions peaked on the day of clutch initiation and strongly declined during egg laying (Fig. 4a). After clutch completion, the breeding pair was rarely seen together. This pattern was similar for the observed copulations (Fig. 4b). Most of these within-pair interactions (325/347) and copulations (63/69) were observed before or during egg laying. After the clutch was complete, and while it was tended by the male, we still observed a few within-pair interactions (11/347) and copulations (3/69; Fig. 4a, indicated in green). The remaining within-pair interactions occurred after the failure of the first clutch (Fig. 4a, indicated in red), and may thus be related to a renesting attempt.

We frequently observed males that interacted with females other than their breeding partner, especially during the laying period of their own clutch, but also afterwards, when their partner had left and they were incubating their clutch (i.e. during incubation breaks; Fig. 4c). Around half of the interactions (74/164) were observed during and after egg laying while the male was paired or tending his clutch (Fig. 4c, indicated in green). These extrapair interactions may indicate a male's attempt to sire extrapair young. The other observed interactions were by males that had lost their clutch (Fig. 4c, indicated in red). These males may have interacted with females with the purpose to renest. Most of the extrapair copulations (9/13, excluding males with a failed nest) were

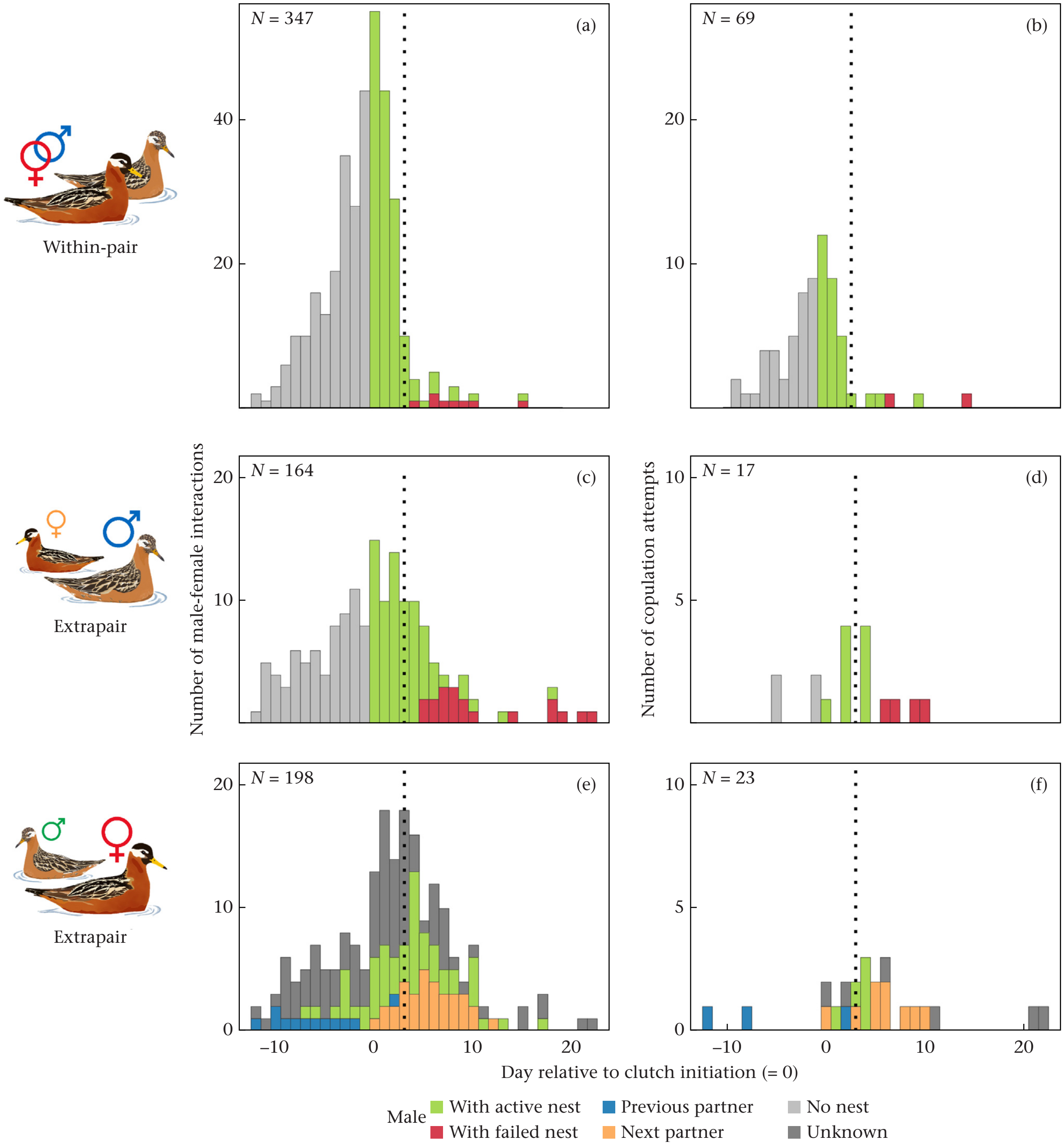

Figure 4. (a, c, e) Number of daily unique male–female interactions and (b, d, f) number of interactions that included at least one copulation that involved breeders on the intensive study plot in 2017–2019 in relation to the initiation date of the focal clutch (i.e. laying date of the first egg equals zero). Interactions are defined as courtship, contact calls, aggression or foraging together (within ca. 5 m). Colours refer to the breeding status of the observed male, related either to the presence of a nest (green: active nest; red: failed nest) or to the focal female (i.e. whether the male was her previous partner (blue) or became her next partner (orange). (a, b) Known breeding partners (breeding pair defined based on observations and genetic parentage, see Methods) interacted with each other before nest initiation (light grey), after nest initiation (green) or after their clutch failed (red). (c, d) Interactions of known male breeders with females other than their social mate before the male's nest initiation (light grey), while their nest had already been initiated (green) or had just failed (red). (e, f) Interactions of known female breeders with males other than their social mate during the focal female's egg-laying period. For many of these extrapair males their breeding status was unknown (dark grey), but some had a known active nest (green). When females had successive breeding attempts, interactions with their next social mate are indicated in orange and those with their previous social mate in blue. Note that these males are not included in the categories for breeding status (unknown or active nest). 'Extrapair' refers to the parents of the focal clutch but does not mean that the social pair bond was still active at the time of the observation. The dotted vertical line indicates the last day of egg laying, assuming a four-egg clutch (mean clutch size = 3.7, SD = 0.6, range 1–4). Sample sizes are shown in the top left corner of each panel.

observed when a male's clutch was almost complete or during early incubation (Fig. 4d), suggesting that this is the main period when males attempt to sire extrapair young.

Females interacted with males other than their breeding partner less often before clutch initiation than during and after egg laying (Fig. 4e). As expected from the short interval between clutches

(Fig. 3c), we observed females interacting with their previous partner up until 2 days before they initiated the next clutch (Fig. 4e, indicated in blue). In one case, we observed a female copulating with her previous partner during the laying period of her subsequent clutch (with a new male), but this copulation did not result in extrapair young.

Six of 11 known polyandrous females interacted with their next breeding partner while laying eggs for their first mate, yet none of these interactions resulted in extrapair offspring in the first clutch (Fig. 4e, indicated in orange), and two of these were observed copulating with their next partner during this time (Fig. 4f, indicated in orange). We observed 14 females during their egg-laying period that interacted with extrapair males that tended a nest of their own (Fig. 4e and f, indicated in green). However, the majority of extrapair males (31/51) with which the focal females interacted during egg laying were without a known active clutch at the time of the interaction and were neither the previous nor the next social mate of the female (Fig. 4e and f, indicated in grey). These males could have been unpaired or may have had an unidentified clutch outside our study site. In addition, some of them may have become the new social mate of the female outside our study site.

DISCUSSION

Previous studies argued that stored sperm from a previous social mate is the main mechanism leading to the occurrence of extrapair paternity in sequentially polyandrous birds (Dale et al., 1999; Oring et al., 1992; Owens et al., 1995; Schamel, Westneat et al., 2004). Yet, evidence for the sperm storage hypothesis remained limited and no alternative mechanisms have been explored. In this study, we tested predictions from the sperm storage hypothesis and considered alternative mechanisms leading to extrapair paternity in the sequentially polyandrous red phalarope (Fig. 1). We have shown that extrapair young can be sired by the female's previous social mate. However, we also showed that most of the identified extrapair sires were not the previous social mate of the female. As predicted from the sperm storage hypothesis, extrapair paternity was more common in the year with the highest rates of polyandry, with second-laid clutches of socially polyandrous females having more extrapair young than first-laid clutches and replacement clutches with the same mate. However, contrary to a prediction from the sperm storage hypothesis, extrapair paternity was not more frequent in clutches laid later in the season. Instead, extrapair paternity mainly occurred during peak egg laying in the local population and may thus be linked to local breeding density. We now discuss each prediction of the sperm storage hypothesis in more detail, evaluate other mechanisms to explain extrapair paternity in red phalaropes, and compare the occurrence of extrapair paternity in other socially monogamous and polyandrous species.

Evaluation of the Sperm Storage Hypothesis

The first prediction was that extrapair paternity should be more common in years with high rates of social polyandry (Oring et al., 1992). Overall, our results suggest that social polyandry and extrapair paternity are relatively rare in the red phalarope with limited variation between years (3–9% of females were polyandrous, and 3–14% of broods contained extrapair young; Fig. 3a). These relatively low rates are in contrast to previously reported high rates of social polyandry in red phalaropes (44%, $N = 9$; Schamel & Tracy, 1977; 50%, $N = 6$; Whitfield, 1995), even though the study of Schamel and Tracy (1977) was done in the same location. The polyandry rates we observed are similar to those reported for the closely related red-necked phalarope (8% over 5 years, $N = 59$; Reynolds, 1987; 9% averaged over 8 years, range 5–16%, sample size unclear; Schamel, Westneat et al., 2004). Nevertheless, the proportion of nests with extrapair young was highest in the year with the highest rate of social polyandry (2019; Fig. 3a), which is in accordance with the first prediction. However, a link between the frequency of social polyandry and extrapair paternity is also predicted from an alternative hypothesis that in years favouring social polyandry, females are more likely to copulate with multiple males (i.e. their social mate, but also others as opportunities arise) to increase their chance of becoming polyandrous, i.e. to acquire another care-giving male.

The between-year differences in the rates of social polyandry may be strongly linked with the length of the breeding season. In both 2017 and 2018, low spring temperatures led to delayed snow melt and consequently a postponed emergence of invertebrates (McGuire et al., 2020) and unusually short breeding seasons (Fig. 3b). In contrast, snow melt occurred much earlier in 2019, allowing phalaropes to nest over a longer period, probably leading to a higher total number of nests and a higher frequency of extrapair paternity, polyandry and renesting (Fig. 3a). This illustrates how in the high Arctic late snow melt in spring constrains the number of breeding birds and the opportunity for females to lay multiple clutches (polyandrous, as well as replacement clutches after clutch predation), in line with observations from previous studies (McGuire et al., 2020; Meltofte et al., 2007; Naves et al., 2008; Oring et al., 1991; Saalfeld et al., 2019). In contrast, polyandrous species breeding in the temperate zone, such as the spotted sandpiper, have a much longer breeding season, allowing females to lay up to six clutches within a season, and 33–100% of the females ($N = 13$ years) are socially polyandrous (Oring et al., 1991, Oring et al., 1992).

The second prediction was that extrapair paternity should be more common in second-laid clutches of socially polyandrous females compared to first-laid clutches and replacement clutches with the same male. Our findings largely support this. None of the first-laid clutches ($N = 15$) and none of the replacement clutches with the same male ($N = 6$) contained extrapair young. In contrast, 27% of the second clutches of socially polyandrous females contained extrapair young ($N = 11$), as well as one of three replacement clutches with mate change (i.e. cases in which a different female laid a clutch for the same male after failure of the previous clutch). We also found extrapair paternity in 10% of single clutches (presumably first clutches) within the intensive study ($N = 138$). However, we cannot exclude that the latter sample includes some second-laid clutches, because females may have moved into the study plot after having laid their first clutch elsewhere. This scenario is supported by the observation of females laying clutches up to 2.2 km apart. Overall, our results are similar to those of Schamel, Westneat et al. (2004), who detected extrapair young in three of six known second clutches from polyandrous red-necked phalaropes, but in none of 25 first clutches and in only one of 16 replacement clutches with the same mate. Thus, in both phalarope species, extrapair paternity occurred more frequently in polyandrous clutches, supporting the prediction of the sperm storage hypothesis.

Red phalaropes are nonterritorial and can move large distances during the breeding season (Krietsch, Valcu et al., 2021). Thus, it is difficult to assign the status of a female (monogamous or polyandrous) or a clutch (first, second or third) within a confined study area. However, if we assume that second clutches of polyandrous females must be laid later in the season, the sperm storage hypothesis predicts that extrapair paternity should mainly occur in clutches laid later in the season (Dale et al., 1999; Owens et al., 1995). Contrary to this prediction, clutches with and without extrapair paternity did not differ in lay date (Fig. 3d). However, our results also show that the assumption that second clutches of polyandrous females are laid later in the season may be invalid when viewed at the population level (Fig. 3c). Polyandrous females initiated their first clutch particularly early in the breeding season (Fig. 3c) and produced their second clutch within a few days after finishing the first. As a result, they laid their second clutch during the peak of egg laying of the season. Thus, lay date cannot be used as an indicator of clutch identity at the population level, except perhaps for the earliest nests, and information on clutch sequence for individual females is necessary to make meaningful predictions about the occurrence of extrapair paternity in relation to clutch order.

The previously discussed predictions only indirectly assess the sperm storage hypothesis but have been used to affirm this hypothesis in the absence of other information. To fully understand the mechanisms behind the occurrence of extrapair paternity, we need to know (1) who sired the extrapair offspring and (2) when the copulations that led to extrapair paternity took place. We found two clutches in which the previous social male sired offspring in the subsequent clutch of the female (Table 2). In both instances the female laid the second clutch immediately (1 and 2 days) after the first clutch had been completed. It is unclear when the female last copulated with the first social mate, but our behavioural observations at the population level showed that within-pair copulations became rare (but did occur) after the second day of egg laying (Fig. 4a and b). This implies that the eggs in the subsequent clutch might have been sired by sperm that was only stored for at most 2–3 days. Therefore, these extrapair offspring could indeed have been the result of within-pair copulations with the previous mate, confirming the sperm storage hypothesis. On the other hand, in seven clutches the extrapair sires were not the previous social mate. These sires were paired to a different social female or were tending a clutch laid by a different female. One of those males sired offspring in a second clutch of a polyandrous female (Table 2). In conclusion, our data provide some evidence supporting the sperm storage hypothesis, but also show that this mechanism is not the only explanation for the occurrence of extrapair paternity in red phalaropes. Even when the previous partner sired offspring in the next clutch of a socially polyandrous female, we cannot rule out that the female copulated with her first mate just before or during egg laying for a second male (i.e. not requiring sperm storage). Indeed, we documented that incubating males copulated with females (to which they were previously mated or others; see Fig. 4b), which indicates that males remain sexually active during this period and which is consistent with the idea that males attempt to sire offspring in the female's subsequent clutch (Dale et al., 1999; Oring et al., 1992).

Interestingly, a reassessment of the results of Oring et al. (1992) on extrapair paternity in the spotted sandpiper suggests that this study provides only minimal support for the sperm storage hypothesis. Oring et al. (1992) showed that two of six (33%) clutches laid after females switched mates contained extrapair young, compared to four of 11 first-laid and same-pair replacement clutches (36%; see Table A3). Furthermore, in only one case was the possible sire of the extrapair offspring the directly preceding mate, while in four instances the possible sire was a previous mate, but the female had laid one to three clutches with other males in between (Table A3). Because sperm is likely to passively leak out of the sperm storage tubules (Birkhead & Biggins, 1998), and because the females must have copulated many times before producing the focal clutch (Colwell & Oring, 1989), fertilizations by sperm more than about a week after the last copulation (up to 31 days, Oring et al. 1992) seem highly unlikely. What seems more plausible is that either the assigned extrapair father was not the sire, or that the male copulated with the female later on, while she was mated with another male (extrapair copulation).

Alternative Mechanisms Explaining Extrapair Paternity

Based on data on the breeding history of the extrapair sires and on observations of the occurrence and timing of male–female interactions, we propose several alternative mechanisms that can explain the observed paternity patterns (Figs. 1 and 4). Our observations show that within-pair interactions (Fig. 4a), as well as copulations (Fig. 4b), peaked at the day of clutch initiation and rapidly decreased during the egg-laying period. During this period, we observed both males and females interacting with extrapair individuals (Fig. 4c–f), a pattern that was also detected in red-necked phalaropes (Schamel, Westneat et al., 2004). This suggests that extrapair paternity might be due to extrapair copulations and to rapid switching between potential mates, and a result of the different tactics males and females use to increase their reproductive success (Fig. 1).

Males can increase their reproductive success in two ways (Fig. 1): (1) by investing heavily in within-pair copulations before and during the laying period of their social female (a) as a paternity assurance mechanism and (b) to increase the likelihood of siring offspring in a potential subsequent clutch of the female; and (2) by seeking copulations with extrapair females, particularly during late laying and early incubation of their clutch. Males might thereby ‘deceive’ females that are looking for a male to incubate a new clutch, pretending to be available although they are mated or on an incubation break.

Females can increase their reproductive success by sequentially procuring care-giving males and producing a clutch for each of them (Fig. 1). Laying the first clutch early in the breeding season (Fig. 3c) and finding another male quickly afterwards, seem to be important for females to become polyandrous. Our observations show that females start to interact and copulate with other males just after clutch initiation and in some cases mate with these males for a second clutch (Fig. 4e and f). Females typically copulated repeatedly with prospective new mates as part of the pair formation process, possibly to ‘convince’ the male that any offspring produced would be likely to be his and thus promote care of their next clutch. Similar behaviours have been observed in spotted sandpipers (Colwell & Oring, 1989) and in red-necked phalaropes (Schamel, Westneat et al., 2004). These observations suggest that males have the highest chance of siring extrapair offspring with females that are in their late egg-laying phase, as these females are more likely to accept copulations as a means to acquiring a subsequent social mate before the end of the season. These copulations might then only lead to fertilizations of the last-laid egg of the current clutch or in a subsequent clutch, given that fertilization of the last egg takes place ca. 24 h before it is laid.

Red phalarope males cannot produce two successful clutches within one short Arctic season (Tracy et al., 2020), but they can renest after clutch failure. We observed that renesting occurred more often with the same than with a different female (6/9 cases), as previously found in red-necked phalaropes (43/65 of cases; Schamel, Lank et al., 2004), and that none of the six clutches where females renested with the same male had extrapair young, compared to one of three after a mate change. Likewise, only one of 16 red-necked phalarope males that renested with the same female lost paternity (Schamel, Westneat et al., 2004). In contrast, in the spotted sandpiper, three of seven clutches of females renesting with the same male had extrapair young (Oring et al., 1992). From the female's perspective, this suggests that it is difficult to find a new mate after having finished laying eggs for the current male. From the male's perspective, it is clearly beneficial to renest with the same female, because the risk of paternity loss to the previous mate will be lower compared to renesting with a new female.

Based on the observed frequency and timing of extrapair paternity, the identity of the extrapair sires and the observed mating behaviour in this study, the role of sperm storage by a previous mate as an explanation for the occurrence of extrapair paternity may be limited. Two of three extrapair young in second clutches of polyandrous females were indeed sired by the previous mate, which might have arisen because the second clutch was produced immediately after the first. However, even in these cases we could not rule out that the female simply continued mating with her initial mate while laying eggs in her second clutch. Further, seven of nine identified sires had their own clutch and fertilized extrapair offspring either while incubating or while their social mate was laying a clutch for them, clearly showing that a mechanism other than sperm storage from the previous mate was responsible for extrapair paternity. Unfortunately, the majority of extrapair sires could not be identified and many of these sires could have been the previous partner of a polyandrous female. However, observations of the timing of interactions of females with males other than their current social mate suggest that extrapair paternity in red phalaropes is likely to be attributable to both extrapair copulations and rapid mate switching.

A few studies on socially monogamous shorebirds also assigned some of the extrapair young to rapid mate switching (Blomqvist, Kempenaers et al., 2002; Mee et al., 2004). However, for most parentage studies in birds, the relative contribution of extrapair copulations versus rapid mate switching to extrapair paternity remains unclear (Ellegren et al., 1995; Ležalová-Piálková, 2011; Sheldon & Ellegren, 1996). Studies that documented cases of rapid mate switching and linked it to patterns of extrapair paternity remain rare (Jacot et al., 2010; Pinxten et al., 1993). Rapid mate switching might contribute more to extrapair paternity in sex role-reversed species compared to socially monogamous shorebirds, because male-only care allows females to rapidly switch to another male.

Oring et al. (1992) and Dale et al. (1999) suggested that earlier-breeding males are of higher quality and that females would thus benefit from storing sperm from their first mate, because it increases the likelihood of having higher-quality offspring. This seems unlikely, because females spent less time with their mate (Krietsch, Valcu et al., 2021) and copulated less frequently (Fig. 4b) later during their laying period. Similarly, Schamel, Westneat et al. (2004) observed that females rejected within-pair copulations in the late egg-laying period in red-necked phalaropes. Instead, our results suggest that as the laying period progresses, females increasingly interacted and copulated with extrapair males, presumably in an attempt to secure a caregiver for a subsequent clutch.

As expected from the patterns of rapid mate switching, but contrary to ideas about the evolution of male-only care, red phalaropes showed higher levels of extrapair paternity (11%, 37/334 of the nests) than most socially monogamous shorebirds with biparental incubation (see Table A1). Similarly, in populations with occasional social polyandry with male-only care, rapid mate switching could explain the occurrence of extrapair paternity in late clutches (Küpper et al., 2004; Maher et al., 2017). In contrast, extrapair paternity was more common in simultaneously polyandrous jacanas, *Jacana jacana* (Emlen et al., 1998; Haig et al., 2003) and black coucals, *Centropus grillii* (Safari & Goymann, 2018), in which females copulate with multiple ‘harem’ males while producing eggs for one of them. Although females in simultaneously and sequentially polyandrous species differ in the strategies to access multiple males, in both groups female behaviour ultimately constrains or circumvents male paternity assurance mechanisms. Females can increase their reproductive success by soliciting copulations from males that can take care of a clutch. Frequent copulations with current mates and prospective new mates are part of this process, which then increases opportunities for males to sire extrapair young.

Author Contributions

J.K., M.V. and B.K. conceptualized the project and the intensive study. R.L. and S.S. conducted fieldwork and contributed the long-term monitoring data. J.K., M.C., B.K, M.V. and S.K. were involved in the field work of the intensive study. M.C. and S.K. performed the molecular analysis. B.K. performed the parentage analysis. J.K. analysed the data with input from B.K., M.V and R.L. J.K. and B.K. wrote the paper with input from R.L. M.C. illustrated the red phalaropes used in the figures. All authors approved the final manuscript.

Data Availability

All data reported in this study, as well as the code used for statistical analysis and figure production, are available in the Open Science Framework (Krietsch, Cragnolini et al., 2021).

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Appendix

Table A1

Frequency of extrapair paternity for monogamous shorebirds with biparental care, compared to polyandrous shorebirds with male-only care

Scientific name	Common name	Nests with EPY (%)	Nests with EPY/total	EPY (%)	EPY/total	Source
Monogamous & biparental care						
Actitis hypoleucos	Common sandpiper	6.7	1/15	1.8	1/53	Blomqvist, Kempenaers et al. (2002)
Actitis hypoleucos	Common sandpiper	18.5	5/27	15.7	13/83	Mee et al. (2004)
Bartramia longicauda	Upland sandpiper	30.4	17/56	15.3	27/177	Casey et al. (2011)
Calidris maritima	Purple sandpiper	3.7	1/27	1.2	1/82	Pierce & Lifjeld (1998)
Calidris mauri	Western sandpiper	7.5	3/40	5.1	5/98	Blomqvist, Andersson et al. (2002)
Charadrius hiaticula	Common ringed plover	0	0/21	0	0/57	Wallander et al. (2001)
Charadrius marginatus	White-fronted plover	0	0/10	0	0/17	Maher et al. (2017)
Charadrius semipalmatus	Semipalmated plover	4.2	1/24	4.7	4/85	Zharikov & Nol et al. (2000)
Charadrius thoracicus	Madagascar plover	0	0/12	0	0/20	Maher et al. (2017)
Haematopus ostralegus	Eurasian oystercatcher	3.8	1/26	1.5	1/65	Heg et al. (1993)
Pluvialis dominica	American golden plover	16.2	6/37	7.6	10/131	Yezerinac et al. (2013)
Polyandrous & male-only care						
Actitis macularius	Spotted sandpiper	20.6	7/34	10.8	12/111	Oring et al. (1992)
Charadrius morinellus	Eurasian dotterel	9.1	2/22	4.5	2/44	Owens et al. (1995)
Phalaropus fulicarius	Red phalarope	33.3	6/18	8.6	6/70	Dale et al. (1999)
Phalaropus lobatus	Red-necked phalarope	6.3	4/63	1.7	4/232	Schamel, Westneat et al. (2004); Schamel, Lank et al. (2004)
Phalaropus tricolor	Wilson's phalarope	0	0/17	0	0/51	Delehanty et al. (1998)
Irediparra gallinacea	Comb-crested jacana	10	1/10	2.8	1/36	Haig et al. (2003)
Jacana jacana	Wattled jacana	24.3	18/74	10.2	24/235	Emlen et al. (1998)

The percentage of nests with extrapair young (EPY) and the total percentage of EPY are shown.

Table A2
Microsatellite loci used for parentage analysis and sexing of red phalaropes, *Phalaropus fulicarius*

Locus	Accession no.	Source	Primer sequences (5′–3′)	C (μM)	Multiplex Mix	Allele size range (bp)	N	N_a	H_{obs}	H_{exp}
ADCYAP1_bm	FJ464427	Steinmeyer et al. (2009)	6FAM-GATGTGAGTAACCAGCCACT ATAACACAGGAGCGGTGA	0.46	3	142–178	2482	28	0.88	0.88
CAM06	–	Dawson et al. (2013)	PET-GTGATGGTCCAGGTCTTGC CAAGAGGAACAGATGAGGGTC	0.76	3	262–290	2477	15	0.75	0.73
CAM17	–	Dawson et al. (2013)	NED-CGGGTTGTAATCAAGAAGATGC CTGCGGAGCAATTAACGC	0.38	2	202–221	2474	20	0.88	0.90
CAM24	–	Dawson et al. (2013)	6FAM-CCCACTTCAGTCTTCAGAGC TGGAGTATTTGGGATTGGAG	0.47	2	109–157	2481	25	0.83	0.84
ClkpolyQ[a]	GQ847510	Johnsen et al. (2007)	6FAM-TTTTCTCAAGGTCAGCAGCTTGT CTGTAGGAACTGTTGYGGKTGCTG	0.33	3	–	–	–	–	–
Cme10	DQ825683	Carter and Kempenaers (2007)	VIC-GAAGGCGAGGAGAACTTCTGT TGTTACCAAAGGCTTAAGCAAAG	0.34	4	172–216	2430	29	0.60	0.81
Cme5	DQ825678	Carter and Kempenaers (2007)	VIC-GTTTACCACACGGCTGCAC CCCCAGCAAGATTTTCTCAT	0.13	3	174–222	2480	21	0.80	0.82
GgaBmaTATC453	DQ173188	Küpper et al. (2008)	NED-CRCATGTTTTGCWGTAGACAA TGTTGATGGAGTAACCAGGA	0.34	1	246–269	2476	16	0.67	0.68
GgaMopl-26	DQ515771	Küpper et al. (2008)	VIC-CAGGAATATAGCTAYCATGCTTAAC GGGSTTTGGTGGTTGAACT	0.19	2	174–180	2477	7	0.52	0.53
GgaRbg18	AY091847	Küpper et al. (2008)	PET-AARTTCAKAAATCTGTTCTGAAAGG TTCCAACTGAGCCCTTGAC	0.89	2	260–294	2478	18	0.83	0.85
GgaRbg27	AY091851	Küpper et al. (2008)	6FAM-TGRCAGGATTGGTCTTGAAAA CCCTYCCAAGAARTCACAGTGAAA	0.38	1	181–209	2480	16	0.36	0.37
GgaSNIPE-B2	AY363298	Küpper et al. (2008)	VIC-ATCTTCCARGCAAATAAATAA AAYTTCACAGTGCAAGGA	0.73	1	157–217	2476	18	0.74	0.76
CcaTgu8	CK309161.1	Olano-Marin et al. (2010)	VIC-GCTCTGCASCAGCCMGAT CCATCAGTTTCCTGTTGCTG	3.60	4	360–384	2473	9	0.60	0.60
CcaTgu9	DV949447.1	Olano-Marin et al. (2010)	6FAM-ACTGGCAGATGGAGGATTTC GGGGATTTTTCAGTCWAAAGA	0.46	3	91–106	2481	16	0.75	0.82
CcaTgu15	DV952837.1	Olano-Marin et al. (2010)	NED-TTAATCCTAGGGTGTYGAGAGAAC CCTTTTTCCTTAAATTAKCTCAGCTT	0.47	1	125–139	2478	15	0.75	0.73
CcaTgu23	CK316117.1	Olano-Marin et al. (2010)	PET-CAAGGMYCATGCCAAAATAA CCCTYCCTCCCTTCAGTTTT	2.17	2	108–135	2480	17	0.38	0.40
CcaTgu7	DV961773.1	Olano-Marin et al. (2010)	VIC-TTTTTCAGGAAARGGAAACA CAAGCTTTTACAGTGCTAWT	1.13	3	273–283	2474	9	0.44	0.44
CcaTgu10	DV575298.1	Olano-Marin et al. (2010)	NED-TGAAAAATCTTGCCTTTTTGGTA TACAAAGCACTGTGGAGCA	0.39	3	136–152	2477	9	0.53	0.54
CcaTgu24[b]	CK316202.1	Olano-Marin et al. (2010)	VIC-ATCTTGAGTYATGACCTTAAAARTCT ATAGCTACAGAAACCTACTTGGGA	–	1	158–173	423	15	0.78	0.85
CcaTgu25	CK305580.1	Olano-Marin et al. (2010)	PET-CCAGGTYRGCATCAGTACAC TAAACCACRCGTGGGCAC	1.99	3	135–165	2481	22	0.74	0.73
CcaTgu26	DV577718.1	Olano-Marin et al. (2010)	PET-AAATTCATTCATRTGATTGGTGG CATATGTACAAAGGCTGMAAAGT	0.55	2	138–151	2469	13	0.74	0.79
CK306783	CK306783	Jakob Müller, personal communication	PET-TGGAATCCTTACCAGCAGTT TGAAACTCAAGMYTGAACCA	0.77	2	180–208	2477	17	0.70	0.72
Tgu07	DV948303.1	Slate et al. (2007)	PET-CTTCCTGCTATAAGGCACAGG AAGTGATCACATTTATTTGAATAT	3.20	1	99–114	2468	16	0.76	0.80
TG11-011	CK308096.1	Dawson et al. (2010)	VIC-ACAAACTAAGTACATCTATATCTGAAG TAAATACAGGCAACATTGG	0.64	2	204–223	2476	14	0.48	0.47
TG01-000	CK314156.1	Dawson et al. (2010)	PET-TTGCTACCARAATGGAATGT TCCTAACCATGAGAAGCAGA	0.54	4	203–220	2477	18	0.88	0.88
LEI160	AM159172.1	Gibbs et al. (1997)	PET-GCAGACAGCCGTTAATATATGCG AACCAAAACACAAGCTCTTGCA	0.60	1	154–174	2481	12	0.38	0.38
Hbaμ4	JQ280749	Williams et al. (2012)	6FAM-GTCCTGCTGGTTTATATC TTCTGCTGAGGTCCTACG	0.51	1	128–170	2482	23	0.88	0.89
Lox1 (PIG)	Y16820	Brownstein et al. (1996); Piertney et al. (1998)	VIC-(gtttctt)ATGATGGTAAGTCTAATGAAAGC CCACACACATTCACTCTATTG	0.51	1	236–261	2477	14	0.44	0.45
Mopl-6	DQ515760	Küpper et al. (2008)	PET-CAATTCAATGGCACTTCCTTCTAAA TCCTTGCCACTTCTGAACACTTATC	0.94	1	283–309	2476	25	0.83	0.90
NPAS2	–	Steinmeyer et al. (2009)	PET-CTGTGGTAAATTTGATGATTCTGA ACACCAAGTTCTTTGCACAATG	0.45	4	164–188	2478	9	0.60	0.61
P2P8[c]	AF006660	Griffiths et al. (1998)	6FAM-CTCCCAAGGATGAGRAAYTG TCTGCATCGCTAAATCCTTT	0.51	2	367–393	2276	9	0.60	0.61
Ppu019	HE616929	Farrell et al. (2012)	6FAM-TAACCCACGAGTGGCTCTG GCTACTGGGTGCTGTTACTTCC	0.34	4	141–179	2468	26	0.82	0.86

Primer sequences include information on fluorescence labels used (6FAM, VIC, PET or NED, Dye Set G5; ThermoFisher Scientific, Waltham, MA, U.S.A.). C: primer concentration in multiplex primer mix to accommodate differences in amplification efficiency and dye strength of the primers (CcaTgu24 excluded). N_a = the total number of alleles, N = the total number of genotyped individuals, H_{obs} = observed heterozygosity, H_e = expected heterozygosity; based on analysis using Cervus 3.0 (see Methods). Cycling conditions for mix 1 were: 15 min initial denaturation at 95 °C; 25 cycles of 30 s denaturation at 94 °C, 90 s annealing at 48 °C and 1 min extension at 72 °C; followed by a 45 min completing final extension at 60 °C. The annealing temperatures for mix 2, 3 and 4 were 52 °C, 55 °C and 56 °C, respectively, with all other conditions the same as described for mix 1.

[a] Not used for parentage analysis.

[b] Only used for the parentage analysis of the 2017 samples.

[c] Only used for sexing, not for parentage analysis.

Table A3

Extended Table 1 of Oring et al. (1992) showing the mating history of spotted sandpiper, *Actitis macularia*, females that produced at least one egg that could not have been sired by the social male that incubated the clutch

Clutch	Female	Male	Clutch complete	No. of eggs	No. of eggs excluded	Possible extrapair sire[a]	After mate change	No. of clutches (and days) in between own clutch and clutch with sired offspring
1990								
1	RA:dBY	dGdG:dGA	30 May	4	1	Unknown		
1991								
1	RA:OB	OR:RA	21 May	4	0			
2		**YA:RO**	**28 May**	**1**	**1**	**OR:RA**	**Yes**	**0 (7)**
3		*YA:RO*	*10 June*	*2*	*0*		*No*	
4		**WA:RY**	**21 June**	**1**	**0**		**Yes**	
5		*WA:RY*	*~3 July*	*4*	*?*	*Unknown*	*No*	
1	MA:GG	OW:OA	24 May	4	0			
2		**dBA:BY**	**31 May**	**4**	**?**	**Unknown**	**Yes**	
3		*dBA:BY*[b]	*12 June*	*2*	*2*	*OW:OA*	*No*	1 (18)
4		*dBA:BY*	*22 June*	*4*	*0*		*No*	
1	OO:dGA	AO:BO	22 May	4	?	Unknown		
2		**GO:WA**		**0**			**Yes**	
3		**dGRdG:RA**	**29 May**	**2**	**?**	**Unknown**	**Yes**	
4		*dGRdG:RA*	*7 June*	*3*	*0*		*No*	
5		**AO:BO**	**13 June**	**3**	**0**		**Yes**	
6		*AO:BO*	*23 June*	*4*	*4*	*GO:WA*	*No*	*3 (?)*
7		*AO:BO*	*~3 July*	*4*	*?*	*Unknown*	*No*	
1	MY:AY	dBR:AG	28 May	3	0			
2		**OR:RA**	**9 June**	**4**	**?**	**Unknown**	**Yes**	
3		**OdB:AdG**	**19 June**	**3**	**0**		**Yes**	
4		**AdG:OdG**	**27 June**	**3**	**1**	**dBR:AG**	**Yes**	**2 (28)**
5		*AdG:OdG*	*~6 July*	*4*	*?*	*Unknown*	*No*	
1	AY:YR	dGRdG:RA	22 May	4	?	Unknown		
2		**GO:WA**		**0**			**Yes**	
3		**MO:OA**	**3 June**	**3**	**0**		**Yes**	
4		*MO:OA*	*13 June*	*3*	*0*		*No*	
5		*MO:OA*	*26 June*	*3*	*2*	*dGRdG:RA*	*No*	*2 (33)*
6		*MO:OA*	*~6 July*	*4*	*?*	*Unknown*	*No*	

Sample sizes of eggs in a clutch, excluded eggs (eggs not sired by the tending male) and the possible extrapair sire are shown. We extended the original table in Oring et al. (1992) by adding whether a focal clutch was produced after a mate change (yes/no) and the number of clutches the female laid in between the clutch she produced with the male that supposedly sired offspring in the focal clutch and this focal clutch, as well as the days between the last egg of this clutch and the first egg of the clutch with the extrapair offspring. Bold indicates females that changed mate for a subsequent clutch; italic indicates females that replaced the removed clutch with the same mate.

[a] Based on multilocus DNA fingerprinting this male could not be excluded as the father. In some cases the sire was unknown because he could not be identified or the clutch could not be analysed (eggs not collected or rotten). Note that details about clutch type are lacking for 17 clutches; in one of these clutches the breeding female was excluded as mother of one young.

[b] Original table says dB:ABY, which is likely to be a typographical error.

Chapter 2

Mutual mate guarding and limited sexual conflict in a sex-role reversed shorebird

Johannes Krietsch, Mihai Valcu, Margherita Cragnolini, Wolfgang Forstmeier and Bart Kempenaers

Mate guarding is typically considered a male strategy to protect paternity. However, under some circumstances, females might also benefit from guarding their mate. Female mate guarding might be particularly important in socially polyandrous species with sex-role reversal, in which females compete for access to care-giving males. Because males also benefit from being near their partner to avoid paternity loss, pair members may have a mutual interest in mate guarding in polyandrous species. We studied the time spent together and movements that lead to separation, as behavioral measures of mate guarding, in the classical socially polyandrous red phalarope (*Phalaropus fulicarius*). We equipped 64 breeding pairs with miniaturized telemetry loggers with GPS to assess variation in mate guarding intensity in relation to breeding phenology and season, nest attendance and the occurrence of extra-pair paternity. We show that red phalarope pairs were almost continuously together in the days before clutch initiation with no sex-bias in separation movements, indicating mutual contribution to mate guarding. Our results suggest that in red phalaropes both pair members guard their mate, with limited sexual conflict arising through biases in the operational sex-ratio and a trade-off with male nest attendance. We found no clear relationship between mate guarding intensity and the occurrence of extra-pair paternity. In this non-territorial socially polyandrous species, mutual mate guarding might be the process underlying the evolution of a brief but strong social pair bond, with no other purpose than producing a clutch for a care-giving male.

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Mutual mate guarding and limited sexual conflict in a sex-role reversed shorebird

Short title

Mate guarding in a sex-role reversed shorebird

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Lay summary

In sex-role reversed species in which males provide all parental care and females compete for males, both pair members benefit from staying close together. Using continuous individual tracking data, we show how the intensity of mate guarding changes before and during egg laying. Mutual mate guarding to avoid paternity loss (male perspective) and mate take-over (female perspective) might be the process underlying the evolution of a brief but strong social pair bond in such species.

Abstract

Mate guarding is typically considered a male strategy to protect paternity. However, under some circumstances, females might also benefit from guarding their mate. Female mate guarding might be particularly important in socially polyandrous species with sex-role reversal, in which females compete for access to care-giving males. Because males also benefit from being near their partner to avoid paternity loss, pair members may have a mutual interest in mate guarding in polyandrous species. We studied the time spent together and movements that lead to separation, as behavioral measures of mate guarding, in the classical socially polyandrous red phalarope (*Phalaropus fulicarius*). We equipped 64 breeding pairs with miniaturized telemetry loggers with GPS to assess variation in mate guarding intensity in relation to breeding phenology and season, nest attendance and the occurrence of extra-pair paternity. We show that red phalarope pairs were almost continuously together in the days before clutch initiation with no sex-bias in separation movements, indicating mutual contribution to mate guarding. Our results suggest that in red phalaropes both pair members guard their mate, with limited sexual conflict arising through biases in the operational sex-ratio and a trade-off with male nest attendance. We found no clear relationship between mate guarding intensity and the occurrence of extra-pair paternity. In this non-territorial socially polyandrous species, mutual mate guarding might be the process underlying the evolution of a brief but strong social pair bond, with no other purpose than producing a clutch for a care-giving male.

Key words

Short-term pair bond, social polyandry, sex-role reversal, extra-pair paternity, sexual selection, *Phalaropus fulicarius*, red phalarope

Introduction

Mate guarding, behaviors that aim at preventing a mate from reproducing with others, has mainly been considered as a manifestation of sexual conflict (Arnqvist and Rowe, 2013; Gowaty, 1996; Parker, 1974; Zuk, 2011). In socially monogamous species, males risk losing paternity if their female engages in extra-pair copulations, which causes selection favoring paternity assurance behavior in males. Male mate guarding is typically characterized by maintaining close proximity to the mate to prevent extra-pair copulations leading to paternity loss, and can be accompanied with or even replaced by frequent within-pair copulation (Birkhead and Moller, 1992; Harts et al., 2016; Møller and Birkhead, 1991). Females may incur a cost of mate guarding (e.g. reduced foraging efficiency; Davis, 2002), and hence might benefit from escaping it, or, alternatively, they may benefit from being guarded by their mate, for example if it leads to a reduced risk of harassment or forced copulation by other males, of contracting a sexually transmitted disease, or of predation (Davis, 2002; Kempenaers et al., 1995; Low, 2005; Rodríguez-Muñoz et al., 2011; Sheldon, 1993).

However, there are also circumstances in which selection can favor females to guard their social mate. For example, in socially facultatively polygynous systems with biparental care, females pay a cost when their mate obtains a secondary female if the male divides his effort between multiple nests (Huk and Winkel, 2006; Schlicht and Kempenaers, 2021; Slagsvold and Lifjeld, 1994). The primary (first-mated) female thus benefits from guarding her mate to avoid or delay the settlement of a secondary female, if this ensures the male's exclusive help with brood care (Kempenaers et al., 1995). Furthermore, mate guarding can be beneficial for both pair members, for example if it reduces the risk of divorce, which can be costly for both sexes in socially monogamous species (Choudhury, 1995). Mutual benefits of mate guarding have also been suggested as an explanation for the evolution of duets in song birds, although this is only one of several alternative hypotheses (Diniz et al., 2020; Grafe and Bitz, 2004; Hall, 2009).

Mutual mate guarding can also be expected in socially polyandrous species, in which patterns of mate guarding have rarely been studied. In this unusual mating system (described for <1% of all species, Cockburn, 2006; Oring, 1986; Owens, 2002), the typical sex roles are reversed, with males providing all parental care. The reproductive rate of females is limited by access to males, leading to competition among females for care-giving males. Thus, females benefit from guarding one (or multiple) males to avoid a take-over by another female. Because females mate with multiple males simultaneously or in close succession, males of socially polyandrous species face a higher risk of paternity loss compared to closely related socially monogamous species (Emlen et al., 1998; Krietsch et al., 2022; Safari and Goymann, 2018; Schamel et al., 2004b).

Thus, males may benefit from guarding their fertile female to avoid paternity loss, while females may also benefit from staying close to their mate if low confidence of paternity would lead to male desertion of the female's clutch. Thus, mate guarding may be mutually beneficial.

Despite these mutual benefits of mate guarding in socially polyandrous species, sexual conflict can arise if trade-offs between mate guarding and other behaviors cause different optima for males and females (Arnqvist and Rowe, 2013). Disentangling the selective forces on male and female mate guarding behavior is therefore challenging and both the social and environmental context has to be taken into account (Haneke-Reinders et al., 2020). One can consider several factors that cause variation in mate guarding intensity in males and females of socially polyandrous species. First, mate guarding is no longer beneficial for a female once her mate is committed to the clutch, because it hinders searching for and mating with a subsequent social male (i.e. a care-giver for a subsequent clutch)(Krietsch et al., 2022). Depending on the timing relative to the end of egg-laying, this can cause a conflict with the female's current mate, because it is in the male's reproductive interest to keep mate guarding until the end of the female's fertile period (i.e. the day on which the penultimate egg is laid; Birkhead and Moller, 1992). This conflict might become stronger as the breeding season progresses, because the number of males tending a clutch increases, which creates a stronger female-biased operational sex ratio and more intense female-female competition for mates (Tracy and Schamel, 1988). Second, male mate guarding would theoretically be beneficial until the end of the female's fertile period, but this may trade-off with nest construction, early incubation or nest protection (Safari and Goymann, 2018). Third, for both sexes, mate guarding might also trade-off with the pursuit of extra-pair copulations (Dickinson, 1997; Hasselquist and Bensch, 1991; Wilson and Swaddle, 2013), although the benefits of extra-pair copulation are less clear for females than for males (Forstmeier et al., 2014). Males may adjust the intensity of mate guarding based on opportunities to sire extra-pair young and on the risk of losing paternity in their own nest, which may both be influenced by male attractiveness (Johnsen and Lifjeld, 1995; Kokko and Morrell, 2005; Wilson and Swaddle, 2013). Females may try to evade mate guarding depending on the benefits of obtaining extra-pair copulations (e.g. fertility insurance). Fourth, in facultatively polyandrous mating systems, mate guarding might also trade-off with the investment into subsequent mates, as previously described in facultatively polygynous mating system (Hasselquist and Bensch, 1991)

We studied variation in the intensity of mate guarding behavior in the sex-role reversed red phalarope (*Phalaropus fulicarius*). In this classical socially polyandrous species, females can lay up to three clutches for different males during a short arctic breeding season, but in our study site most females only laid one clutch (Krietsch et al., 2022; Schamel and Tracy, 1977). Based on location data from 64 red phalarope breeding pairs, we describe two behavioral measures related to mate guarding: (1) the proportion of time the pair spent in close proximity, and (2) the number of movements initiated by the male or the female that clearly separate the pair members, as well as the distance of these flights (Gowaty, 1996; Parker, 1974).

The first aim of our study was to assess whether mate guarding is mainly driven by males as a paternity protection behavior, by females to retain a care-giver for her clutch, or by both pair members (mutually beneficial). If mate guarding is mainly male driven, we predict that pairs should be in close proximity most of the time during the female's fertile period, and that males should not (or rarely) initiate movements resulting in separation of the pair during this period. In contrast, if mate guarding is mostly driven by females, we expect that pairs should stay in close proximity immediately after pair formation until the male is committed to tend his mate's clutch, which does not necessarily coincide with the end of the female fertile period. Furthermore, females should not (or rarely) initiate movements during this period which result in separation from the mate. Mate guarding could therefore be mutually beneficial for both sexes, until the fertile female starts pursuing other mating opportunities, i.e. attempts to attract a care-giver for a subsequent clutch (Krietsch et al., 2022).

The second aim of our study was to test whether mate guarding intensity increases over the season, and whether polyandrous females or early breeding females with potential to become polyandrous associate less with their mate during egg laying. If mate guarding is female driven and related to the risk of losing the care-giving male, we expect that mate guarding intensity will show seasonal changes, being less intense early in the season when most males are available and stronger when some males start incubating, leading to a more female-biased operational sex ratio. During the short Arctic breeding season the probability of producing multiple clutches declines steeply and only early breeding females have a chance to become polyandrous (Krietsch et al., 2022). Thus, early in the season, selection might favor females to quickly move on to attract a new partner, while later in the season, when the opportunity to find a new mate is low, females might benefit from associating longer with their current mate, to potentially quickly lay a replacement clutch, should their first clutch fail.

The third aim of our study was to assess whether the intensity of male mate guarding declines, because the male starts spending more time at the nest. In red phalaropes, the nest is a simple scrape on the tundra, but males add a bit of nest material (small leaves) and create some cover by bending grasses over the nest (Mayfield, 1979). Moreover, males might need to guard (cover) the nest once the first egg appeared to avoid egg predation (Smith et al., 2012).

The fourth aim of our study was to investigate whether mate guarding intensity during the fertile period differed between males that lost paternity and those that did not. If mate guarding is male driven and related to paternity protection, mate guarding intensity might be linked to the occurrence of extra-pair paternity. Males that lost paternity might have guarded less intensely (i.e. extra-pair paternity is a consequence of a lack of mate guarding) or they might have guarded more intensely (if there was an indication that their mate was seeking extra-pair copulations, making the best-of-a-bad-job (Kempenaers et al., 1995; Kokko and Morrell, 2005).

Methods

Study species and study site

We studied red phalaropes (*Phalaropus fulicarius*) in a 2.5 km^2 plot of open wet tundra habitat near Utqiaġvik (formerly Barrow), Alaska (71°19'N 156°39'W) between late May and late July 2018 and 2019. Red phalaropes are non-territorial, sex-role reversed, sequentially polyandrous birds that breed circumpolar in the high Arctic (Tracy et al., 2020). Females do not defend resources, but compete for males (Schamel and Tracy, 1977). Red phalaropes form short-term pair bonds that are characterized by close proximity between female and male, frequent within-pair copulations, and frequent communication with contact calls (Kistchinski, 1975; Schamel and Tracy, 1977). An earlier study showed that 33% of clutches (6/18) contained at least one extra-pair offspring (Dale et al., 1999), but a recent study with a larger sample size found a substantially lower rate (11% of clutches, 37/334) with yearly variation between 0% and 19% (n = 8 years; Krietsch et al., 2022). Previous studies also suggested that the frequency of sequential polyandry was high (proportion of females that laid clutches for >1 male: 44% (4/9) females; Schamel and Tracy, 1977); and 50% (3/6 females; Whitfield, 1995). However, a recent, more intensive study found a much lower rate, with on average 7% of females (11/162, range: 3-9% over 3 years) laying clutches for multiple males (Krietsch et al., 2022).

Field procedures

We caught red phalaropes with handheld mist nests as soon as they arrived in the study site (total number of individuals: 203 in 2018 and 319 in 2019). Each captured individual was sexed based on plumage characteristics (Tracy et al., 2020), and banded with a U.S. Geological Survey metal band and a unique combination of four color bands that allowed us to identify and document the behavior of individual birds. The assigned sex of each individual was later confirmed with molecular methods (Krietsch et al., 2022).

After pairs were observed, we searched for nests daily across the study site with a team of 2-10 people by (a) following males or pairs that made scrapes or sat in scrapes, behavior typically shown a few days before the first egg was laid, (b) following females until they went to a scrape or nest to lay an egg, (c) following males after flushing them off a nest (accidentally or by rope-dragging) or during a natural incubation break until they returned to their nest, and (d) by inspecting potential nest locations based on the GPS data of males (see below). Then, to avoid loss of data through predation, we collected all eggs and replaced them with dummy eggs that resembled real eggs in size, weight and color. The collected eggs were hatched in an incubator, and the young brought back to an incubating male after blood sampling. This allowed us to determine parentage (based on 30 microsatellite markers) of almost all eggs laid within the study site and of some within the surrounding area (for details see Krietsch et al., 2022).

We determined clutch initiation dates (i.e. the date the first egg was laid) either by (1) subtracting one day for each egg in the nest for clutches found during egg-laying (assuming one egg per day was laid, 41% of nests), (2) subtracting one day for each egg in the clutch plus the mean incubation period of 17 days (0.8 SD; range: 15-20 days) for eggs hatching in the incubator (based on 58 nests with known laying date) or 19 days for naturally incubated clutches (Weiser et al., 2018) (34% of nests), or (3) subtracting the estimated developmental age based on flotation (Liebezeit et al., 2007) and clutch size for clutches that did not hatch (12% of nests), (4) by correcting dates estimated with method (2 or 3) with conflicting yet more reliable field observations (the number of eggs in the clutch; 10% of nests), or (5) based on nest visits by tagged males (3% of nests). For females known to lay multiple clutches (based on parentage analysis), we categorized each clutch as first, second, or third based on clutch initiation date.

All procedures were approved by the US Geological Survey Bird Banding Laboratory (permit number 23520), the Alaska Department of Fish and Game (permit numbers 17-149, 18-146 and 19-143), the US Fish and Wildlife Service (permit number MB210494-0), and the North Slope Borough and Ukpeaġvik Iñupiat Corporation.

Tracking methods and data processing

We attached 3.5 g NanoRadioTag-3 (Milsar Inc.) telemetry loggers with a solar panel on the back of some of the caught birds within the study area (2018: N = 101 of 210 caught in total, 53 males and 48 females, 2019: N = 202 of 323 caught individuals, 105 males and 97 females). Four males where equipped with a tag in both years and three individuals received a second tag after the first one fell off. We focused on early-arriving birds and attached the majority of tags before the peak of clutch initiation (median attachment date: 14 June in 2018 (range: 11-22 June) and 10 June in 2019 (range: 5-27 June); median clutch initiation date: 23 June in 2018 (N = 37 clutches) and 14 June in 2019 (N = 100)). In 2018, we glued the tag, mounted on a piece of goatskin, on the back of the bird after cutting some body feathers. We used a flexible, highadherence, non-toxic glue (Pattex Repair Extreme). In 12 cases, we either found the tag on the tundra or we observed the bird without a tag after on average 5 days of attachment (range: 1-11 days). Therefore, in 2019, we used a backpack harness made out of medical-grade silicon tubing (DKA-795, Reichelt Chemietechnik GmbH). To assure that the harness would eventually fall off, we connected the tag with the harness material using a single string of dissolvable sewing material (Novosyn Quick) as a weak link. No bird lost its tag during the study period in 2019.

Each tag was set to provide a GPS position every 10 min, with data stored on the tag until they were transmitted to a base station. We downloaded data daily for individuals that stayed within the study area, using a handheld base station with an omnidirectional antenna (~500 m range) or with a fixed directional antenna (~1500 m range) on a high pole. To maximize downloading data from individuals that had left the study area, we drove along the roads around Utqiaġvik once a day with a directional antenna mounted to a van. In 2019, we additionally downloaded data by flying a drone (Dji Mavic 2 Pro) with an attached omnidirectional antenna (~1000 m range) in or near the study area.

Overall, we retrieved data from 296 out of 303 tagged individuals (100 in 2018 and 196 in 2019). The seven remaining tags (one in 2018 and six in 2019) never downloaded, most likely because these individuals had left the study area soon after release. In 2018, we obtained locations for up to 27 days (median = 4) and in 2019 for up to 51 days (median = 11). The difference between the years was due to the change in attachment method and to the shorter breeding season in 2018 (Krietsch et al., 2022). In 2018, we found 13 nests with a tagged male and 13 nests with a tagged female, with both individuals tagged for 11 nests. In 2019, we found 76 nests with a tagged male and 66 nests with a tagged female, with both individuals tagged at 56 nests (four of these nests were social pairs renesting after failure of the first clutch).

To assess variation in mate guarding intensity, we used location data from 64 social pairs between the pre-laying and the end of the laying period (68 nests, 10 from 2018 and 58 from 2019). For the four pairs that produced two clutches (first and replacement clutch), we assigned all data until the failure of the first clutch to the first clutch and all data thereafter to the second clutch.

For each of the breeding individuals, we first selected all location data from the moment the individual had been released until the last data had been sent or until the individual had reached the position where the tag fell off. We then filtered out implausible positions, that is, (1) those >500 km away from the capture site, 821 of 359,547 positions, 0.2%), (2) those that implied a faster speed than the maximum speed recorded in a continuous track (>105 km/h, 27 positions, <0.01%) and (3) single outliers (one location >2.5 km away from previous and subsequent positions, which were within 100 m, excluding 23 positions, <0.01%). Thus, we used a filtered dataset of 358,676 positions.

Data analysis

All data were analyzed with R version 4.2.2 (R Core Team, 2023) using RStudio (RStudio Team, 2023). We fitted generalized linear mixed models (GLMMs) using the package "glmmTMB" (Brooks et al., 2017) with a binomial distribution and logit link when the dependent variable was binary, with a beta-binomial distribution and logit link when the dependent variable was a proportion and with a Gaussian distribution when the dependent variable was continuous. In all models, we included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. We assessed model residual diagnostics using the package "DHARMa" (Hartig, 2022). We extracted effect sizes using the package "effects" (Fox and Weisberg, 2018). We present back-transformed mean effect sizes with standard error (SE) in the text and full model summaries in the supplementary material. All figures were created using the package "ggplot2" (Wickham, 2016).

Time spent together

We used the filtered location data to determine when pair members or any two opposite-sex individuals were together, as follows. First, we calculated for each male-female pair the distances between locations that were recorded simultaneously, i.e. within maximally 10 min of each other (in 95% of cases this time gap was within 5 min of each other, median 2.6 min). Then we used three rules to define whether two individuals were "together" at a given time. Two individuals were defined as being together if, first, the distance between their locations was smaller than a dynamic threshold of 30 m or more (the dynamic threshold allows for movements that happen during the time gap mentioned above; details described in Figure S1), and, second, in a bout of successive locations "together" (below the dynamic threshold) at least one distance was smaller than 30 m (fixed threshold). If the last locations of the pair members in a bout of "together" observations were > 30 m apart, we defined the two individuals as "not together" in this last instance before separating. The latter was necessary, to distinguish flights that resulted in separation from flights that the birds did together (being together once the flight has come to an end; Figure S1). Taken together, these three rules yielded a classification that corresponds with our intuitive judgment (see supplementary video). The proportion of time spent together on a given day was then calculated as the number of locations where the pair members were "together" divided by the total number of recorded locations for the pair on that day.

To assess the effect of phenology and season on the time spent together, we fitted GLMMs with the proportion of time spent together per day as the dependent variable and day relative to clutch initiation (i.e. the day on which the first egg was laid = 0) and clutch initiation date (the actual date, standardized by subtracting the mean of each year) as explanatory variables. In all models, day relative to clutch initiation and clutch initiation date were fit first as covariates with a quadratic effect. When the quadratic effect was non-significant, we only included the linear effect in the final model. Because the birds' behavior may change after the start of egg laying, we fitted separate models for the period before clutch initiation (day -5 to -1) and during egg laying (day 0 to 3, most females laid 4 eggs). We initially tested for daily variation by fitting both models with the binary variable "together" (yes/no at a given 10-min interval) as the dependent variable and time (as sinus and cosinus) as additional explanatory variable. Daily variation in the time spent together was biologically irrelevant in the period before clutch initiation (daily maximum minus daily minimum = 1%; Table S1), but more pronounced during egg-laying (max-min = 10%; Table S2), with birds spending more time together midday (71%; ± 3.6 SE) compared to midnight (61%; ± 4.1 SE). Note that during the study period there is 24 h day light and birds are active around the clock (Steiger et al., 2013). Because we were interested in seasonal variation and because the models using data at 10 min intervals suffer from temporal autocorrelation, we used the proportion of time spent together per day in subsequent models. We also fit year as an additional explanatory variable, but we excluded it in the final model because there were no significant differences in the proportion of time spent together between the years for both periods (day -5 to -1: p = 0.30; day 0 to 3: p = 0.83; Table S3 and S4; Figure S3).

We compared the proportion of time spent together by breeding pairs and by non-breeding "random" pairs (two opposite-sex individuals that did not breed together), using the same two models, but including pair type (breeding pair or random pair) in interaction with day relative to clutch initiation and clutch initiation date as explanatory variable. We fitted an additional model for the period after the egg-laying period (days 4 to 10) with the same structure. For the random pairs, we only included pairs of opposite-sex individuals if (1) we had location data for at least 50% of a given day and (2) the pair had at least one 10 min period "together". The latter excludes pairs of individuals that were never in each other's neighborhood (e.g. using the same foraging site). To obtain comparable data given variation relative to clutch initiation of the breeding pairs, we randomly sampled 50 non-breeding pairs for each day relative to the clutch initiation date of each focal female.

Movements away from the mate

We defined the individual that was responsible for separating the pair as the individual that moved the furthest between two 10-min periods with a change from “together” to “not together”, as defined above. Note that this criterion may not always identify the correct individual that left the partner, because individuals can move undetected within the 10-min interval. However, in 72% of the cases when a pair split, one individual stayed stationary (i.e. within 30 m) and in the other 28% of the cases the individual that was assigned as responsible for the separation moved on average 64 m (median) further than the other pair member.

We used all separation events and fitted GLMMs with the binary variable “female move” (yes/no, whereby “no” means that the male was responsible for the separating move) as the dependent variable and with day relative to clutch initiation and clutch initiation date as explanatory variables. Again, we fitted separate models for the period before clutch initiation (days -5 to -1) and during egg laying (day 0 to 3). Additionally, we fitted a generalized linear mixed model with the distance moved away when separated as the dependent variable and with the same explanatory variables, but also including the sex of the moving bird.

Time spent at the nest with and without the mate

We defined an individual as “at the nest” if either (1) its position was within 15 m of the known nest coordinates or (2) when the individual was “together” with the partner, and the partner was within 15 m of the nest. For each day, we then calculated the proportion of positions an individual was at the nest, with or without its mate, and overall. We also determined the proportion of the day the focal individual was not together with its mate and not at the nest.

For males and females separately, we fitted GLMMs with the proportion of time spent at the nest per day during the period of egg-laying (day 0 to 3) as the dependent variable and day relative to clutch initiation and clutch initiation date as explanatory variables.

Mate guarding behavior in relation to extra-pair paternity and social polyandry

We evaluated whether social pairs with extra-pair sired eggs in their clutch differed in mate guarding behavior (time spent together and proportion of separating moves by the male or the female) from pairs without extra-pair paternity. We fitted GLMMs with the proportion of time spent together per day or “female move” (yes/no, whereby “no” indicates that the male was responsible for the separation) as the dependent variable and extra-pair paternity (yes/no) and day relative to clutch initiation and clutch initiation date as explanatory variables. We fitted separate models for the potential fertile period of the female: before clutch initiation (day -5 to -1) and during clutch initiation (day 0 to 2, excluding the last day of laying).

As our sample size of polyandrous females was small, we only visually compared the time the female spent together with her first and second mate relative to the initiation of the two clutches.

Results

Time spent together in relation to clutch initiation and season

We caught and observed most red phalaropes in pairs, suggesting that individuals were already paired before or soon after they arrived at the study site. The tag data show that members of a future breeding pair were often together almost immediately after tag deployment and release (Figure S4). Before clutch initiation, breeding pairs typically spent most of their time together (percentage of time together from day -5 to -1: 90 % ± 2.2 SE; Table S5 and Figure 1a) and moved together between potential foraging or nesting sites (see supplementary videos of three pairs as examples). The proportion of time spent together peaked two days before clutch initiation with 34 out of 40 breeding pairs for which we had data on this day spending more than 90% of the time together (94 % ± 1.4 SE; Figure 1a). When egg laying started the time spent together was still high (day 0: 84% ± 2.1 SE), but it decreased rapidly throughout egg-laying (day 3: 28% ± 4.9 SE; Table S6). Throughout the pre-laying period, as well as during egg-laying, breeding pairs spent much more time together than random pairs of opposite-sex individuals that were at least once together but did not breed together (breeding pairs day -5 to -1: 94% ± 0.7 SE; randomized pairs: 12% ± 1.4 SE, $p < 0.001$, Table S7; breeding pairs day 0 to 3: 58% ± 3.2 SE; randomized pairs: 11% ± 1.5 SE, $p < 0.001$; Figure 1a, Table S8). In the days after clutch completion, breeding pairs spent little time together, and as little as random pairs (breeding pairs day 4 to 10: 10% ± 1.1 SE; randomized pairs: 11% ± 1.2 SE, $p = 0.13$, Table S9; Figure 1a).

The proportion of time breeding pairs spent together was not only changing with the day relative to clutch initiation, but was also influenced by the season (i.e. the actual date at which the clutch was initiated). In the pre-laying period (days -5 to -1), breeding pairs spent most time together (> 90%) in clutches that were laid in the middle of the season (between one day before and seven days after the mean clutch initiation date of a given year; Figure 1b). Breeding pairs that laid earlier or later than this period spent less time together (six to two days before the mean clutch initiation date: 74% ± 6.6 SE; eight to twelve days after the mean: 73% ± 8.6 SE; Table S5, Figure 1b). A similar pattern was found in the laying period (days 0 to 3), when breeding pairs that laid their first egg one day after the mean clutch initiation date spent most time together (Table S6, Figure 1c).

Movements away from the mate in relation to clutch initiation and season

Given that pairs spent most of the time together before clutch initiation, they only separated rarely in this period (day -5 to -1; mean by pair and day: 1.9 times, range: 0 to 9 times, Figure S5). Pair separation became more frequent during egg-laying (day 0 to 3; mean by pair and day: 4.6 times, range: 0 to 17 times). Males and females were more or less equally responsible for movements that resulted in the separation of the pair both before and during laying (proportion of separating moves made by the female, day -5 to -1: 45% ± 5.0 SE; day 0 to 3: 52% ± 3.3 SE; Table S10 and S11, Figure 2a).

However, separation movements varied across the season, at least during the pre-laying period. In pairs that initiated laying early in the season, separating movements were mostly initiated by females (59% ± 6.2 SE, pairs with clutch initiation before the mean), while for later pairs, separations were mostly initiated by males (35% ± 5.5 SE female initiated, pairs with clutch initiation on or after the mean laying date, $p = 0.01$; Table S10, Figure 2b). The effect of clutch initiation date was less clear and not statistically significant for movements during the laying period (47% ± 4.3 SE vs. 58% ± 4.8 SE, $p = 0.19$; Table S11, Figure 2c).

During both the pre-laying and the laying period, the distance of the movement that led to the partners being separated was independent of the sex of the individual responsible for the movement and only slightly larger during laying (days -5 to -1: males: 140 m ± 15.2 SE, females:170 m ± 15.8 SE, $p = 0.07$; Table S12; days 0 to 3: males: 194 m ± 25.8 SE, females: 216 m ± 25.8 SE, $p = 0.16$; Table S13, Figure S8).

Time spent at the nest with and without the mate

Most red phalarope pairs only started spending time at their nest location three days before clutch initiation (mean: 2.6 days before clutch initiation, range: -7 to -1; N = 28 pairs with at least one day of data before the day of the first nest visit). On the day before clutch initiation, both sexes spent around 10% of their time at the nest (Figure 3a). On the day of clutch initiation, females spent the longest time at the nest overall (median: 16 % of their total time), but this time decreases rapidly over the laying period to 7% on the last day of laying; Figure 3a). In contrast, males spent an increasing amount of time at the nest (from 20% on day 0 to 66% on day 3), suggesting a gradual start of incubation (Figure 3a).

How much time males and females spent at the nest was also influenced by the timing of clutch initiation within the season. Males whose female started laying relatively early in the season spent less time at the nest during the laying period (before or at the date of mean clutch initiation: 34% ± 3.5 SE; Table S14, Figure 3b) compared to males whose female laid later in the season (after the mean clutch initiation date: 44% ± 4.4 SE). Females spent somewhat less time at the nest in early and late clutches, with a "peak" around the mean clutch initiation date (Table S15, Figure 3c).

During the egg laying period, males and females spent an increasing amount of time separated from their partner (Figure 1a). Males that were away from their partner spent most of this time at the nest (Figure 3d), suggesting a potential trade-off between nest attendance and mate guarding. Conversely, if females were alone, they were virtually always away from the nest (Figure 3e).

Mate guarding behavior in relation to extra-pair paternity and social polyandry

During the pre-laying period, breeding pairs with extra-pair paternity (at least one extra-pair sired egg in the clutch) spent a similar amount of time together compared to pairs without extra-pair paternity (Figure 4a, Table S16 and Figure S7a). In the fertile period during egg-laying, pairs with extra-pair paternity spent somewhat more time together than those without, but the difference is not significant ($p = 0.22$, Figure 4a, Table S17).

In pairs with extra-pair paternity, females moved away from their mate more often than the other way around before clutch initiation, but this difference was not significant ($p = 0.13$, Figure 4b, Table S18 and Figure S7b). In the fertile period during egg-laying, there were no differences between the sexes (Figure 4b, see also Table S19).

We obtained location data from four polyandrous females and both of their social mates. One female spent more than 60% of her time together with the first mate before switching abruptly to the second mate after the completion of her first clutch (Figure 5a). The other three polyandrous females already spent some time together with the second mate while or even before egg-laying for the first clutch (Figure 5b-d).

For three socially polyandrous females (Figure 5a-c) we had location data from her and her first mate during the laying of the second clutch. These data show that those females still spent some time in close proximity to their first social mate during this period, despite the fact that these males were incubating the first (and not the second) clutch.

Discussion

In this study of a socially polyandrous, sex-role reversed species, we found multiple indications suggesting that both sexes guard their mate in a context-dependent manner, with females likely guarding males to ensure a care taker for their clutch and males guarding females to ensure their paternity. Mate guarding was most intense in the days just before the female laid the first egg and during the population-wide peak of clutch initiation when competition for mates was presumably the strongest (Figure 1). During the female's laying period, the association between the male and the female rapidly dissolved, with males spending more time at the nest and females searching for subsequent males (Figure 3). We found no clear link between mate guarding intensity and the occurrence of extra-pair paternity, but only few clutches contained extra-pair sired eggs (Figure 4).

Our study suggests mutual mate guarding in red phalaropes particularly before clutch initiation. Red phalaropes spent almost all the time together with their mate and on average there was no sex-bias in separation movements (Figure 1 and 2). While high-intensity mate guarding has also been described for many socially monogamous bird species (e.g. Birkhead, 1979; Gowaty and Plissner, 1987; Hoi et al., 2011; Johnsen and Lifjeld, 1995; Komdeur, 2001; Pinxten et al., 1987), the observed patterns are typically driven by the behavior of the male. In these species, the male typically follows his fertile female and the majority of separation events are initiated by the female. Intense mate guarding has also been described in the closely related red-necked phalarope *Phalaropus lobatus*, which is also socially polyandrous with male-only care (Schamel et al., 2004b). In this study, behavioral observations showed that males moved more often towards the female than the other way around, but both sexes were equally likely to move away from their mate. However, Schamel et al. (2004b) only discussed mate guarding from the male perspective as a paternity protection behavior.

Several behavioral observations of social pairs (see Krietsch et al., 2022) provide additional support that in phalaropes both males and females actively guard their mate. (1) In 153 cases in which the sex that initiated the flight could clearly be identified, 58% (88/153) of flights were initiated by the female and in 82% (72/88) of those cases, the male followed his female. Males initiated 42% (65/153) of the flights and in 75% (49/65) of the cases the female immediately followed her partner. (2) We recorded 144 aggressive interactions of pair members towards one or more other individuals. Aggressive interactions were initiated slightly more by females compared to males (males: 43%, 62/144; females: 57%, 82/144; both pair members: 6%, 9/144). Both male and female aggression was mainly directed towards another female (males: 66%, 40/61; females: 57%, 46/80), and less often against another male (males: 41%, 25/61; females: 42%, 34/80). The observation that the pair (i.e. both pair members) responded aggressively towards other individuals that approached them, which was also observed by Schamel and Tracy (1977) and also in red-necked phalaropes (Tracy and Schamel, 1988), suggests that they signal unavailability to other potential partners. (4) As reported for red-necked phalaropes (Schamel et al., 2004b), red phalarope pairs copulated frequently and copulations were initiated by both sexes. In 113 cases in which the initiating sex could be unequivocally identified, the male initiated the copulation with a "whirr-flight" in 82 cases (73%), while the female first lifted her tail and crouched in the other 31 cases. (5) Pair members use contact calls whenever direct visual contact is lost (Tracy et al., 2020). Frequent use of contact calls in phalaropes may reflect mutual mate guarding, as has been hypothesized for the evolution of duet songs in some birds (Diniz et al., 2020; Grafe and Bitz, 2004; Hall, 2009), although in territorial species it is difficult to distinguish from mutual territory defense. Because red phalaropes are non-territorial, this alternative hypothesis can be excluded.

We found no clear relationship between mate guarding intensity and the occurrence of extra-pair paternity. Pairs with extra-pair paternity spent a similar amount of time together before clutch initiation and slightly more time together during laying (non-significant), compared to pairs without extra-pair paternity, but extra-pair paternity was rare (Figure 4). In the period before clutch initiation, females that had extra-pair sired eggs were responsible for somewhat more separation movements, but again the difference was not significant. During laying both pair members initiated an equal proportion of separation movements. Low rates of extra-pair paternity in the population (11% of nests (37/334); Krietsch et al., 2022) suggest that female red phalaropes do not typically try to escape male mate guarding. Previous observations also suggested that red phalarope females rarely interact with extra-pair males and they were never seen copulating outside the pair bond before clutch initiation (Krietsch et al., 2022).

Mate guarding was most intense during the peak clutch initiation period, when the conditions were presumably best for breeding and competition for mates likely the strongest. Early in the breeding season, pair members spent less time together before clutch initiation and males were less likely to leave their female during this period (Figure 1 and 2), suggesting that mate guarding at this time could be more male driven. This pattern could be due to a male-biased sex-ratio early in the season, with more intense male-male competition for females (Tracy and Schamel, 1988). With the progression of the season, each female that completes a (first) clutch will immediately re-join the mating pool, whereas their male partner starts incubation and is therefore excluded from the mating pool. Therefore, over time, the operational sex-ratio will become more female biased and female-female competition should become more intense (Lank et al., 1985; Schamel et al., 2004a; Tracy and Schamel, 1988). These changes in mate availability could explain why later in the breeding season, pairs spent less time together before clutch initiation and separation movements become more male-biased (Figure 1 and 2), suggesting that mate guarding becomes more female driven. Males, as the only providers of parental care, may also have a decreasing interest in accepting a clutch, because of the lower prospect of chick survival later in the season (Saalfeld et al., 2021). Thus, seasonal effects on the operational sex ratio and on the likelihood of successful breeding can affect male and female investment in mate guarding.

After egg-laying started the close association between pair members rapidly dissolved (Figure 1). During this period, separation movements showed no sex bias, suggesting little sexual conflict (Figure 2). During the day of clutch initiation, most pairs were still together more than 80% and both partners mainly visited the nest location together. Thereafter, males increasingly spent time alone at the nest, while females mostly foraged or interacted with a potential subsequent partner away from the nest (Figure 5). Male mate guarding, from a paternity assurance perspective, should be continued until extra-pair copulations can no longer fertilize an egg, which should be up to the day on which the penultimate egg is laid (Birkhead and Moller, 1992). However, mate guarding intensity clearly decreased earlier, suggesting a potential trade-off for males between mate guarding and nest attendance. Nest attendance might become important because of nest building activity or to reduce predation risk (i.e. by reducing exposure of the eggs), as has been shown for other species (Safari and Goymann, 2018; Schleicher et al., 1993). Alternatively, the probability that copulations during the egg-laying period lead to fertilization of an egg in the clutch might already be low, which would also explain why within-pair copulations become less frequent once laying starts (Krietsch et al., 2022). From the female's perspective, the benefits of mate guarding might also be lower after clutch initiation, if the probability of male desertion is low once egg-laying has started. Females might then leave their social partner to increase the probability of finding a subsequent mate. Indeed, behavioral observations show that red phalarope females strongly increase the amount of extra-pair interactions and extra-pair copulations directly after clutch initiation, which can lead to a rapid subsequent reproductive event (Krietsch et al., 2022). This could also explain why pairs spent less time together after laying has started earlier in the season, because during that period females are more likely to find another mate and become socially polyandrous. Hence, females breeding earlier in the season might invest more in finding a second mate compared to females laying later in the season. After the population peak in clutch initiation, when female-female competition for a few remaining males is likely strongest, females might benefit from spending more time with the current mate, for example to reduce male harassment by other females around the nest (Kistchinski, 1975). In accordance with this hypothesis, both sexes spent more time at the nest later in the breeding season (Figure 3c and 3d).

In conclusion, in the socially polyandrous sex-role reversed red phalarope, both pair members may benefit from mate guarding. Males would benefit from guarding their mate to protect their paternity, while females need to secure a male that cares for their clutch. To achieve this, females need to deter competitors (to avoid mate take-over), but they might also need to assure the male that he sired the eggs (to avoid male desertion). These male and female-specific reproductive interests would create the circumstances in which an exceptionally short and intense social pair bond with affiliative behavior and mutual mate guarding could have evolved. Mutual interest in staying close to the mate seems particularly strong during the peak breeding season, but the potential for sexual conflict might increase early and late in the breeding season. This seasonal effect is likely linked to shifts in the operational sex-ratio, which creates a situation in which the less common sex becomes more valuable for the more common sex. Mate guarding has primarily been studied from the male perspective as a paternity-guarding mechanism, and disentangling the selective forces on male and female behavior in a "shared trait" is challenging. In this study, we show that to understand variation in mate guarding behavior it is important to consider both the male and female perspective, as well as context-dependent changes in reproductive interests of both males and females.

Data and code availability

All data reported in this study, as well as the code used for data analysis, statistical analysis and figure production, are available in the Open Science Framework (Krietsch et al., 2023).

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Author Contributions

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Project administration (supporting); Writing – review and editing (supporting). **Margherita Cragnolini**: Methodology (supporting); Investigation (supporting); Project administration (supporting); Writing – review and editing (supporting). **Wolfgang Forstmeier**: Conceptualization (supporting); Formal analysis (supporting); Writing – review and editing (supporting); Visualization (supporting). **Bart Kempenaers**: Conceptualization (equal); Methodology (supporting); Formal analysis (supporting); Investigation (supporting); Resources (lead); Writing – original draft (supporting); Writing – review and editing (lead); Visualization (supporting); Supervision (lead); Project administration (equal), Funding acquisition (lead).

Figures

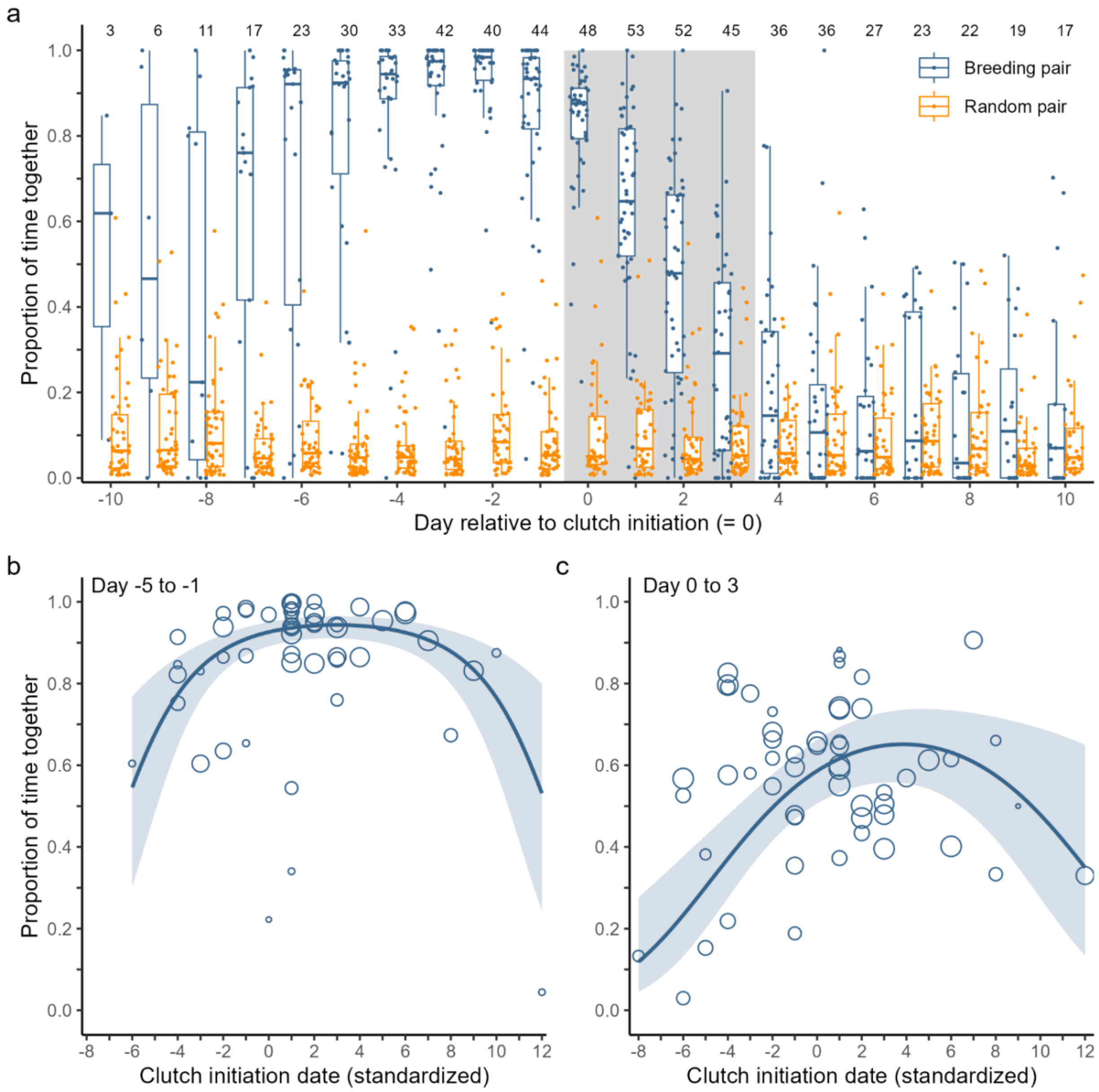

Figure 1. (a) Proportion of time red phalarope breeding pair members spent together in relation to day relative to the initiation of the clutch of the focal pair, and compared to random (non-breeding) pairs of opposite sex individuals. The data are from a total of 64 breeding pairs (68 clutches) and 50 random pairs (see methods). Shown are box plots with the median (center line), 25–75th percentile (limits), minimum and maximum values without outliers (whiskers), and raw data for each day by breeding pair (dots). The grey shaded area indicates the egg laying period, assuming a typical clutch of four eggs. Numbers on top indicate the number of pairs with data for each day. (b, c) Proportion of time red phalarope breeding pair members spent together in relation to their date of clutch initiation, standardized by subtracting the mean of each year,

during the pre-laying period (b) and the laying period (c). Model estimates (lines), 95% confidence intervals (shaded areas) and the mean proportion by pair (dots) show that early and late-breeding pairs spent less time together than those breeding in the middle of the season. Dot size reflects the number of pairwise observations for each breeding pair (range: 14-686 observations). See Table S5 and S6 for model descriptions.

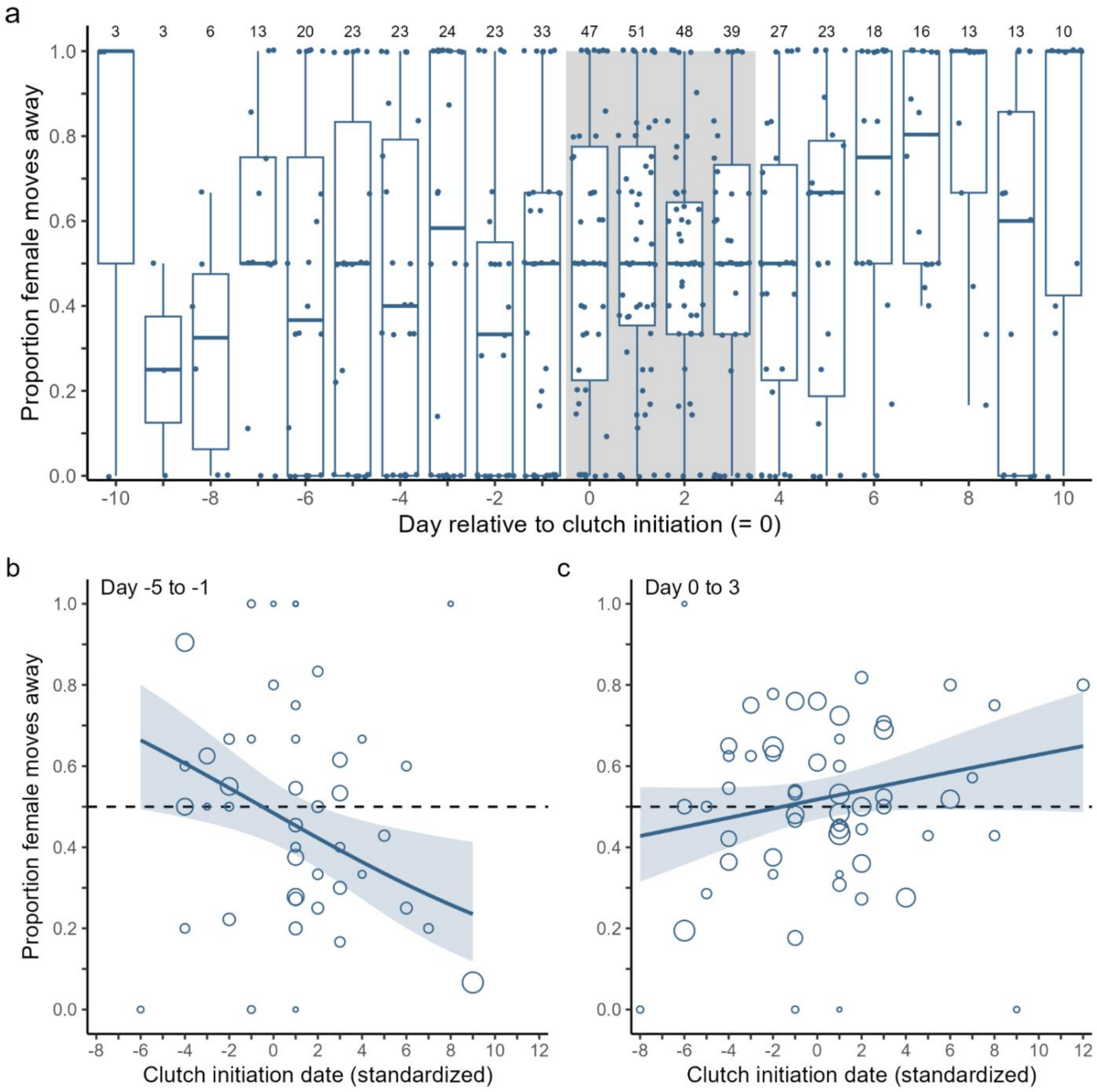

Figure 2. (a) Proportion of the total number of separating flights in which the female moved away from the male in relation to the start of egg laying, i.e. day relative to the initiation of the clutch of the focal pair. Shown are box plots with the median (center line), 25–75th percentile (limits), minimum and maximum values without outliers (whiskers), and raw data for each day by breeding pair (dots). The grey shaded area indicates the egg laying period, assuming a typical clutch of four eggs. Numbers on top indicate the number of pairs with data for each day. (b, c) Proportion of the total number of separating flights in which the female moved away from the male in relation to the clutch initiation date (standardized by subtracting the mean of each year), during the pre-laying period (b) and the laying period (c). Shown are model estimates (lines), 95% confidence intervals (shaded areas) and the mean proportion by pair (dots). Dot

size reflects the number of pairwise observations for each breeding pair (range: 1-37 observations). See Table S10 and S11 for model descriptions.

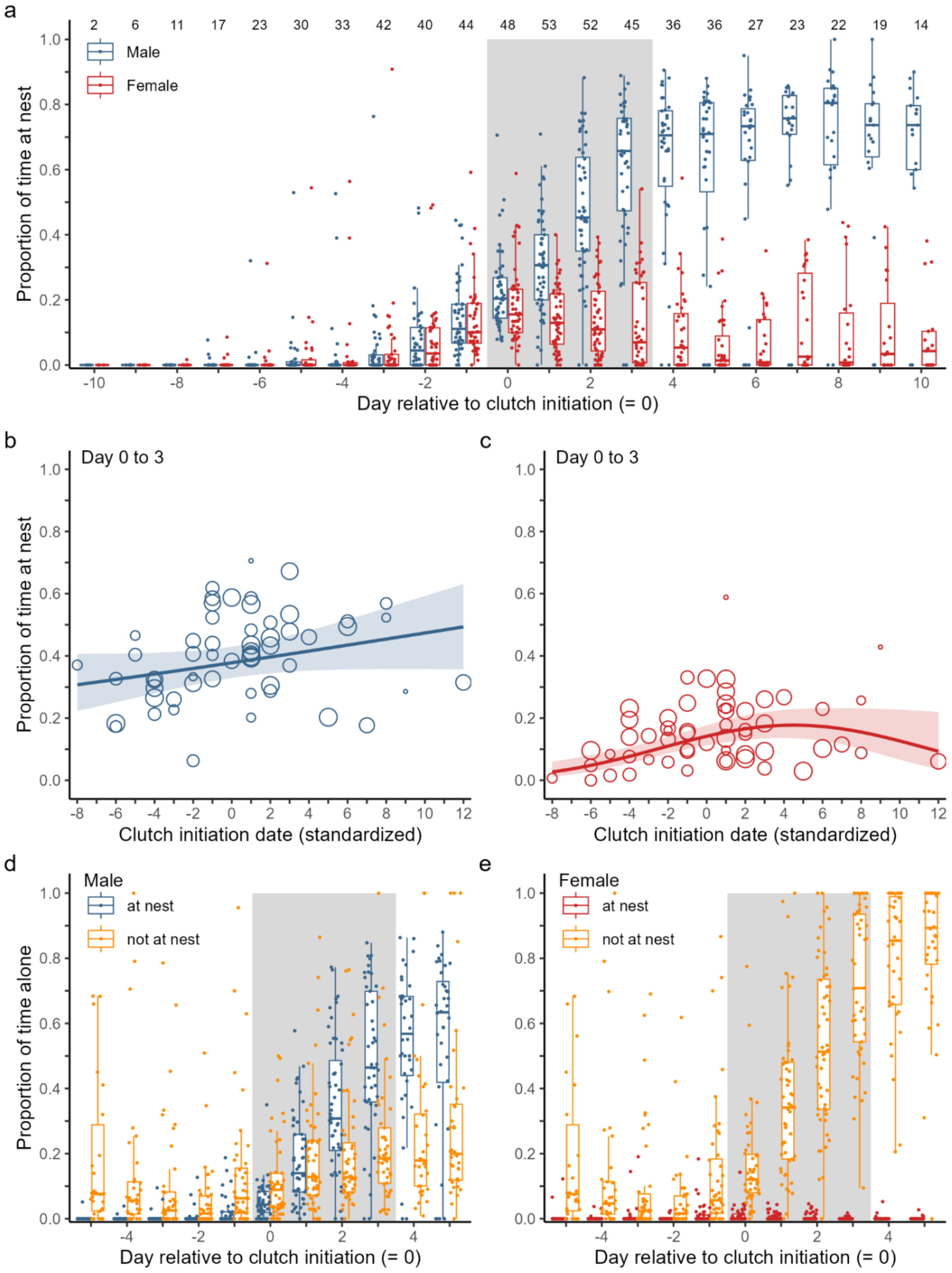
a
2 6 11 17 23 30 33 42 40 44 48 53 52 45 36 36 27 23 22 19 14
Male
Female
Proportion of time at nest
1.0
0.8
0.6
0.4
0.2
0.0
-10 -8 -6 -4 -2 0 2 4 6 8 10
Day relative to clutch initiation (= 0)
b
Day 0 to 3
Proportion of time at nest
-8 -6 -4 -2 0 2 4 6 8 10 12
Clutch initiation date (standardized)
c
Day 0 to 3
-8 -6 -4 -2 0 2 4 6 8 10 12
Clutch initiation date (standardized)
d
Male
at nest
not at nest
Proportion of time alone
-4 -2 0 2 4
Day relative to clutch initiation (= 0)
e
Female
at nest
not at nest
-4 -2 0 2 4
Day relative to clutch initiation (= 0)

Figure 3. (a) Proportion of time male and female red phalaropes spent at the nest (location within 15 m or together with mate that is within 15 m from the nest) in relation to the start of egg laying, i.e. day relative to the initiation of the clutch of the focal pair. Shown are box plots with the median (center line), 25–75th percentile (limits), minimum and maximum values without outliers (whiskers), and raw data for each day by sex for each breeding pair (dots). The grey shaded area indicates the egg laying period, assuming a typical clutch of four eggs. Numbers on top indicate the number of pairs with data for each day. (b, c) Proportion of time male (b) and female (c) red phalaropes spend at the nest in relation to the clutch initiation date (standardized by subtracting the mean of each year). Shown are model estimates (lines), 95% confidence intervals (shaded areas) and the mean proportion for each sex (dots). Dot size reflects the number of observations for each sex (range: 14-558 observations). See Table S14 and S15 for model descriptions. (d, e) Proportion of time male and female red phalaropes spend alone at the nest and away from the nest in relation to egg date. Shown as in (a).

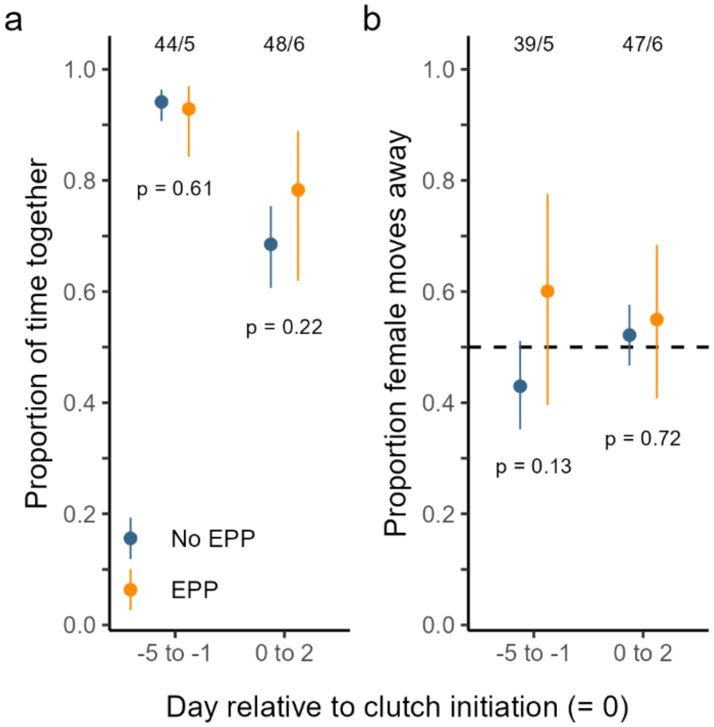

Figure 4. (a) Proportion of time red phalarope breeding pair members spent together during the fertile period of the female for pairs with and without extra-pair paternity (EPP, defined as whether at least one egg in the clutch was sired by an extra-pair male). (b) Proportion of the total number of separating flights in which the female moved away from the male for pairs with and without extra-pair paternity. Shown are model estimates and 95% confidence intervals for the pre-laying period (days -5 to -1) and for the laying period during which the female was still fertile (days 0 to 2). Numbers on top indicate sample sizes (the number of pairs in each category). P-values for each comparison are given below the estimates. See Table S16-S19 for model descriptions.

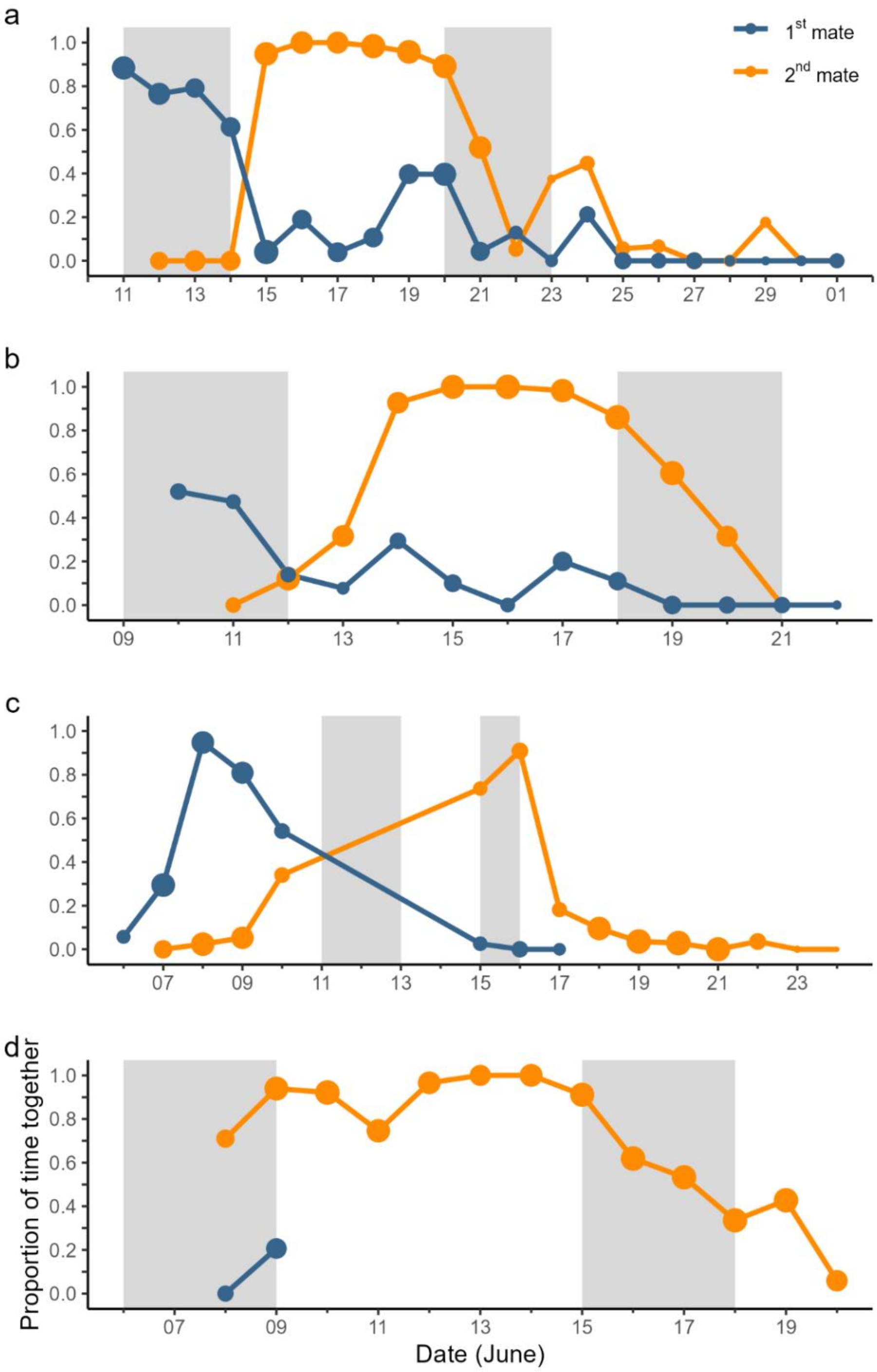

Figure 5. The proportion of time a polyandrous red phalarope female (N = 4, a-d) spent together with her first (blue) and second (orange) social mate across the season. The grey shaded areas indicate the egg laying periods for the first and second clutch. Clutch size was four, except for the female in (c), who laid only three and two eggs, respectively. Dot size reflects the number of observations (10-min periods; range: 1-142).

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Chapter 2 - Supplementary material

Mutual mate guarding and limited sexual conflict in a sex-role reversed shorebird

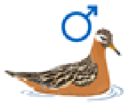
Johannes Krietsch, Mihai Valcu, Margherita Cragnolini, Wolfgang Forstmeier and Bart Kempenaers

Supplementary video

https://www.youtube.com/watch?v=hJtalnXZ498

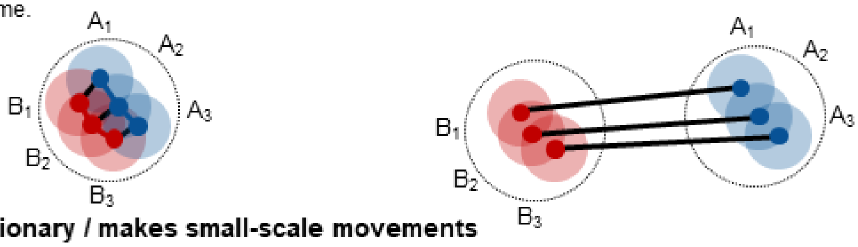
Visualization of movements before and during clutch initiation of three red phalarope pairs (0:00 first pair, 2:48 second pair, 5:14 third pair). Females are indicated in red, males in blue and the nest location in brown (turning dark with clutch initiation). The moving bar on top indicates 12 hours before and 12 hours after the present (black bar in the middle); birds classified as together are shown in green and those classified as not together in white. Periods with missing pair-wise data are indicated in grey. On the map, a green ring around the present location (dots) indicates when pair members were classified as together. Eggs appear at the top left around the time when they were approximately laid. Note the running date and time and distance between the pair at the bottom right, and the scale bar at the bottom left. Beige indicates land (i.e. tundra habitat), light blue lakes, darker blue the sea, and grey buildings and roads. Map data are from OpenStreetMap in polar Lambert azimuthal equal area projection with longitude origin 156.65° W (Utqiaġvik).

Supplementary figures



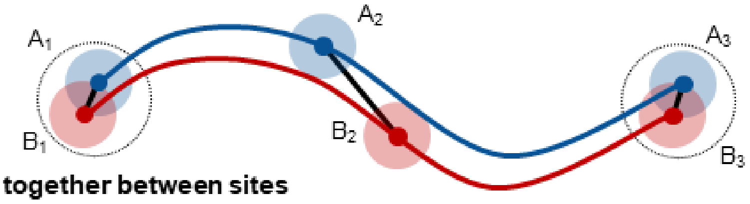
Interpretation of bouts of subsequent locations

Each of the four scenarios below show three locations for the male (blue, A_1-A_3) and the female (red, B_1-B_3) of a pair, that were recorded in 10-min intervals, with a 3-min delay for the female locations. The red and blue lines illustrate the actual track of the two individuals. The light red or light blue area around each point indicate a 15 m radius (level of accuracy) and the black lines show the distance between the male and female locations at a given time interval (e.g. $d(A_1B_1)$). The dotted circles represent different sites where the birds spent a longer amount of time.



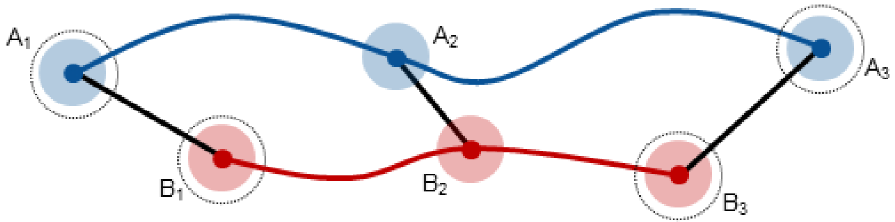
① **Pair is stationary / makes small-scale movements**

If males and females stay more or less stationary during the 3 subsequent 10-min intervals, a 30 m threshold would be sufficient to classify birds as "together" (left) or "not together" (right).



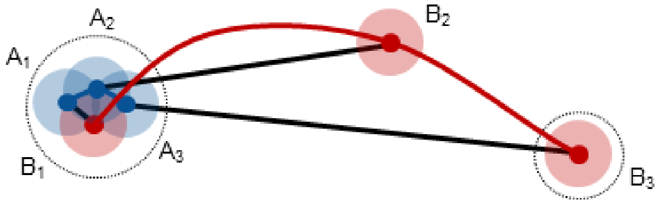
② **Pair moves together between sites**

A simple threshold incorrectly classifies the pairwise locations A_2 and B_2 as "not together", because these locations were recorded 3-min apart. A dynamic threshold of $d(A_2B_2) < [30\text{ m} + d(A_1A_2) + d(B_1B_2)]$ classifies them correctly as "together".



③ **Pair members separately move between sites**

A dynamic threshold misclassifies the pairwise locations A_2 and B_2 as "together", but as none of the pairwise positions were within 30 m the whole bout is classified as "not together".



④ **Pair members separate**

A dynamic threshold misclassifies the pairwise locations A_2 and B_2 as "together", but this is corrected given that the birds are not together during the next time interval (A_3, B_3).

Figure S1. Graphical illustration of different scenarios to define whether two birds (e.g. a breeding pair) were "together" or "not together" based on three subsequent GPS locations (points) from a male (A_1 to A_3) and a female (B_1 to B_3) red phalarope. GPS fixes were typically obtained at 10-min intervals. When a pair was caught together, we started their tags at the exact same time, but in general the fixes were not synchronised between the two individuals. In the illustrated example, the female GPS locations were recorded 3 min after the male GPS locations. Thus, we first defined "pair-wise" locations as any two GPS fixes recorded closest in time between the two individuals, excluding any that were more than 10 min apart (here: A_1B_1, A_2B_2 A_3B_3). In our dataset, 50% of pair-wise male-female positions were within 2.6 min and 95% within 5 min. In scenario ①, the birds stayed together in the same site (dotted circle, left) or they stayed separately at two different sites (right) over time. In this scenario, we can then define birds as "together" or "not together" based on a fixed threshold of 30 m (i.e. locations within 30 m are classified as together, $d(AB) < 30$ m), as illustrated by the overlap in the 15 m light red and light blue area around the points. This threshold was based on data on tag accuracy (Figure S2), as well as direct observations of breeding pairs showing that they usually stay within 15 m of each other when they are together, i.e. they were in visual and acoustical contact (with frequent low amplitude contact calls). However, using a fixed threshold leads to false negative ("not together") classifications when birds move together, as illustrated in scenario ②. In this example, the pair moved together (blue and red lines) from one site (A_1B_1) to a different site (A_3B_3). Using a fixed threshold would classify the pair as "together" at the beginning and end of the flight when they were stationary (because $d(A_1B_1)$ and $d(A_3B_3) < 30$ m), but as "not together" during the flight, because the female's position was recorded 3 min after the male's position (e.g. if the birds would have moved in one direction at a speed of 15 km/h, a 3 min delay in fixes would locate the female 750 m from the male, such that $d(A_2B_2) = 750$ m). Therefore, we used a "dynamic threshold" to define locations as "together" or not. This dynamic threshold includes the fixed threshold of 30 m, but additionally takes the distances that were moved by each individual between the previous and the current position into account. Thus, the birds are classified as "together" when $d(A_2B_2) < [30 \text{ m} + d(A_1A_2) + d(B_1B_2)]$. However, this dynamic threshold is too lenient, because it can lead to false positives (i.e. it misclassifies individuals as together when they are in fact separated), as illustrated in scenario ③. To correct these misclassifications, we used the additional rule that each bout of being "together" (a continuum of pair-wise locations classified as "together", separated by pair-wise locations that are classified as "not together") needs to contain at least one instance where the distance between the two birds was smaller than the fixed threshold (i.e. $d(A_i,B_i) < 30$m). Otherwise, the entire bout is classified as birds being "not together". This approach leaves one last problem unsolved, which is illustrated in scenario ④. When one or both birds fly away from each other after having been together, the dynamic threshold will still classify the birds as “together” (e.g. A_2B_2) because the bird that left is still within the dynamic threshold distance, even though they are no longer together. To solve this issue, we defined the last pairwise location in a bout of “together” observations as “not together” when it was above the fixed threshold of 30 m. The procedure described here yielded a classification that corresponds to our intuitive judgment (see supplementary video).

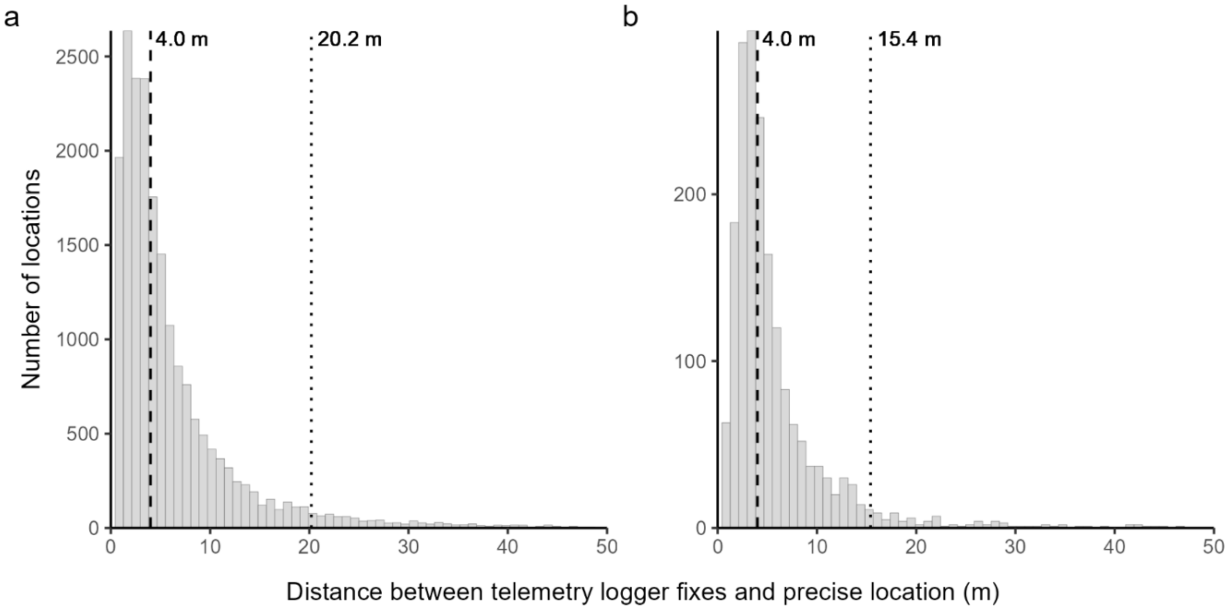


Figure S2. Illustration of the accuracy of the NanoRadioTag-3 (Milsar Inc.) telemetry loggers. Shown are the distances between the loggers' GPS fixes and the precise known locations. (a) Data from 10 loggers that were placed in our study site at a fixed location for 15 days. Of a total of 20150 recorded locations, 95% were within 20.2 m of the precise location, and the median distance was 4.0 m. Overall, 0.7% (N = 133) of the recorded locations were > 50 m away from the precise location (maximum = 398 m) and these were excluded from the plot. (b) Data from one male red phalarope with a logger while incubating (exact location of nest known). We extracted periods of incubation based on data from a temperature logger placed in the nest (using a 30°C threshold). Of a total of 1846 positions recorded during incubation, 95% were within 15.4 m of the precise nest location and the median distance was 4.0 m. Overall, 0.3% (N = 5) of the recorded incubation positions were > 50 m from the nest (max = 90 m) and these were excluded from the plot. At each fixed location or nest, we determined the precise coordinates using waypoint averaging for 10 min with a Garmin Oregon 700.

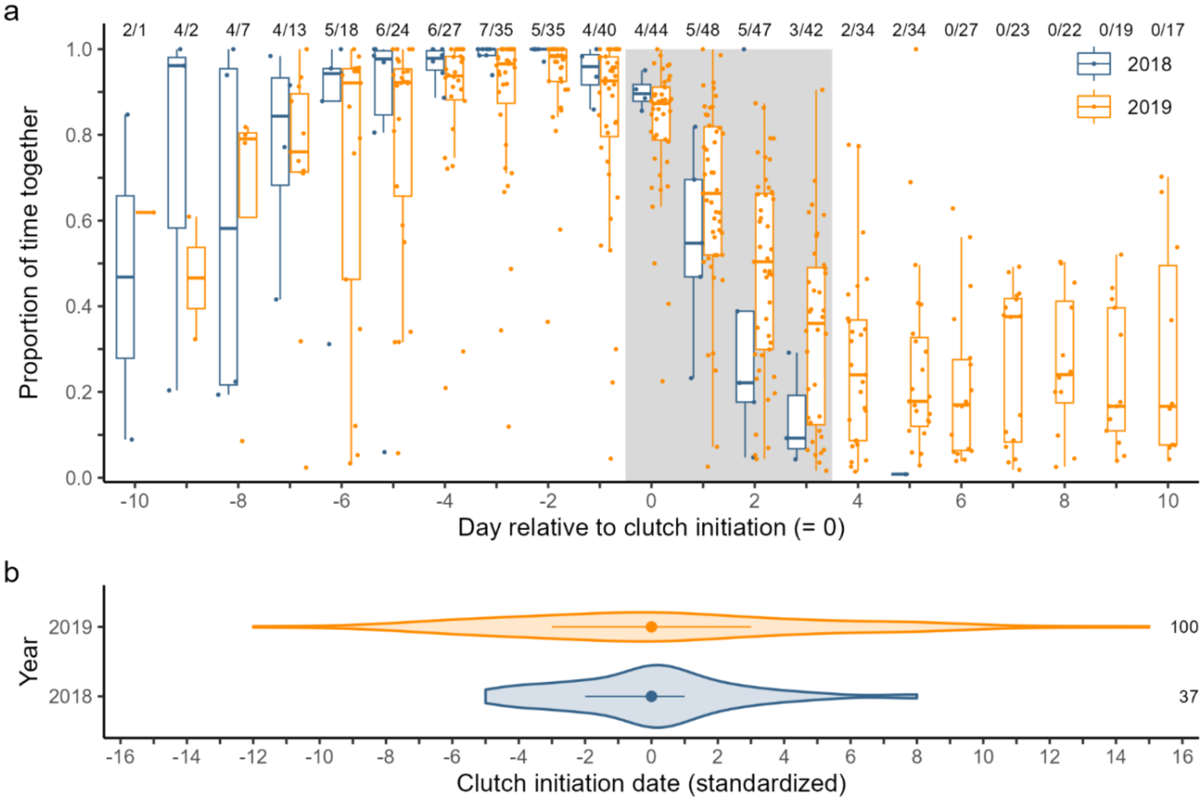


Figure S3. (a) Proportion of time male and female red phalaropes spent together in 2018 and 2019 in relation to the start of egg laying, i.e. day relative to the initiation of the clutch of the focal pair. The data are from a total of 64 breeding pairs (68 clutches). Shown are box plots with the median (centre line), 25–75th percentile (limits), minimum and maximum values without outliers (whiskers), and raw data for each day by breeding pair (dots). The grey shaded area indicates the egg laying period, assuming a typical clutch of four eggs. Numbers on top indicate the number of nests with data for each day and year. (b) Distribution of clutch initiation dates (standardized by subtracting the mean of each year) within the intensive study plot (see methods) for 2018 and 2019. Shown are the median (dot), 25–75th percentile (lines) and a violin plot illustrating kernel probability densities, i.e. the width of the grey area represents the proportion of data located there (maximum width: 11 nests in 2018 and 10 nests in 2019). The difference in the length of the breeding season between the years was due to an exceptionally late snow melt in 2018.

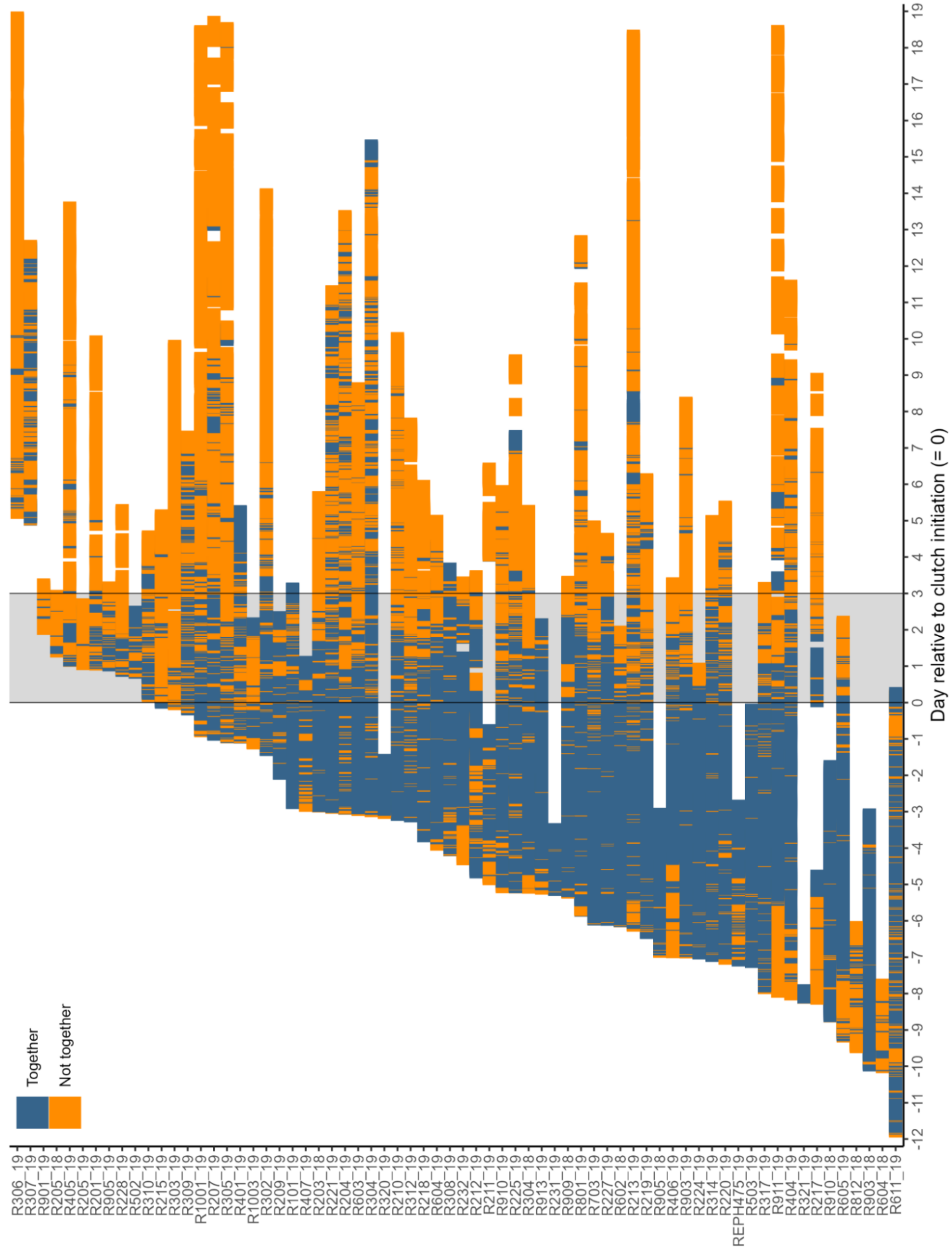

Together
Not together
Nest
Day relative to clutch initiation (= 0)
-12
-11
-10
-9
-8
-7
-6
-5
-4
-3
-2
-1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
R306_19
R307_19
R901_18
R205_18
R405_19
R205_19
R201_19
R905_19
R228_19
R502_19
R310_19
R215_19
R303_19
R309_19
R1001_19
R207_19
R305_19
R401_19
R1003_19
R302_19
R209_19
R101_19
R407_19
R203_18
R221_19
R204_19
R603_19
R304_19
R320_19
R210_19
R312_19
R218_19
R604_19
R308_19
R232_19
R212_19
R211_19
R910_19
R225_19
R304_18
R913_19
R231_19
R909_18
R801_19
R703_19
R227_19
R602_18
R213_19
R219_19
R905_18
R406_19
R903_19
R224_19
R314_19
R220_19
REPH475_19
R503_19
R317_19
R911_19
R404_19
R321_19
R217_19
R910_18
R605_19
R812_18
R903_18
R604_18
R611_19

Figure S4. Overview showing whether the members of a red phalarope breeding pair were together or not for each of 68 nests (from 64 breeding pairs). Data are shown for each 10 min-period in relation to the start of egg laying, i.e. the day relative to the initiation of the clutch of the focal pair. The grey shaded area indicates the egg laying period, assuming a typical clutch of four eggs. Each nest is represented by a bar and white indicates missing data.

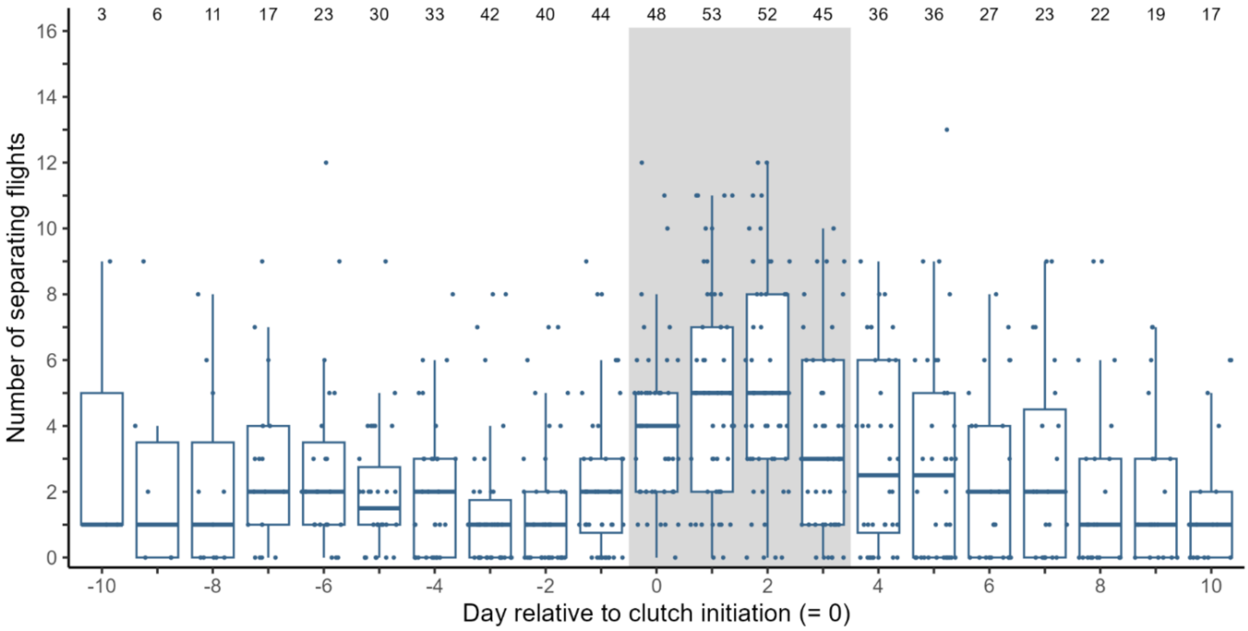


Figure S5. Number of separating flights in which the female or the male moved away from its mate in relation to the start of egg laying, i.e. day relative to the initiation of the clutch of the focal pair. Shown are box plots with the median (centre line), 25–75th percentile (limits), minimum and maximum values without outliers (whiskers), and raw data for each day by breeding pair (dots). The grey shaded area indicates the egg laying period, assuming a typical clutch of four eggs. Numbers on top indicate the number of nests with data for each day.

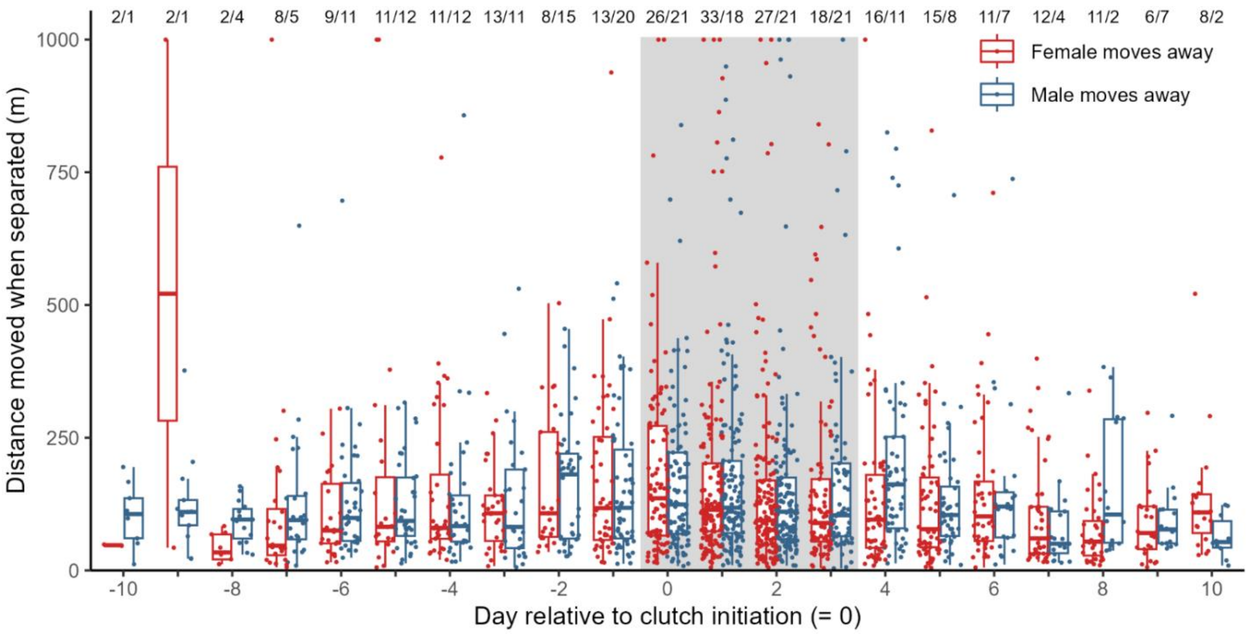


Figure S6. Distances moved by the sex that was responsible for the separation of the breeding pair in relation to the start of egg laying, i.e. day relative to the initiation of the clutch of the focal pair. Box plots show the median (centre line), 25–75th percentile (limits), minimum and maximum values without outliers (whiskers), and raw data for each day and breeding pair (dots). Distances > 1000 m were set to 1000 m.

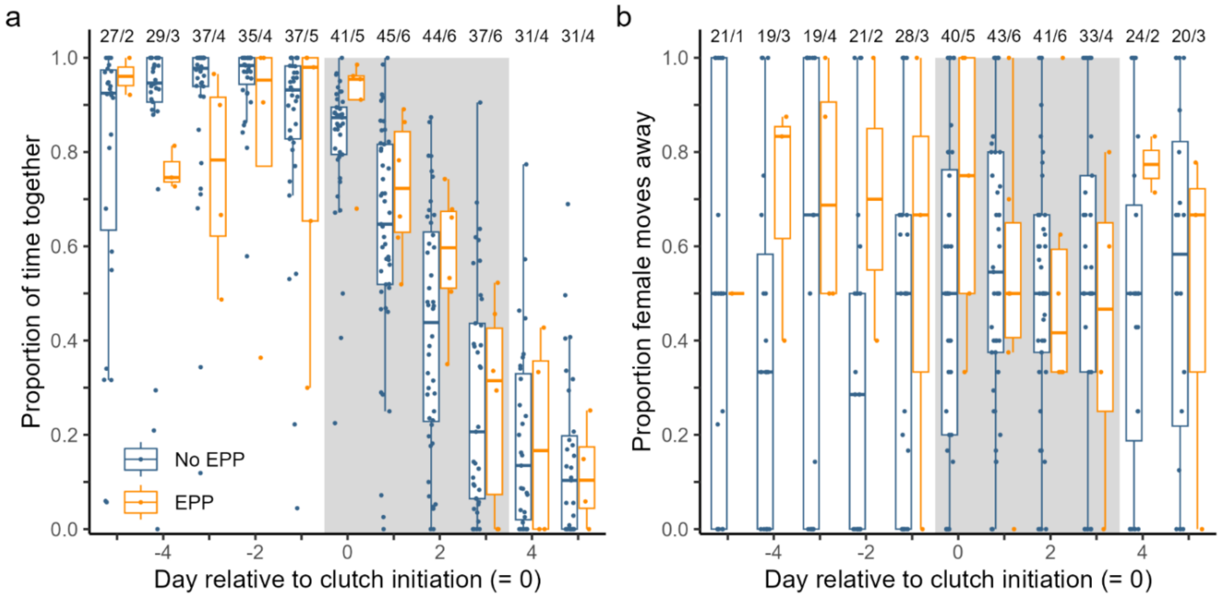


Figure S7. (a) Proportion of time red phalarope pair members spent together in relation to the start of egg laying (i.e. day relative to the initiation of the clutch of the focal pair) for pairs with and without extra-pair paternity (EPP; i.e. whether at least one egg in the focal clutch was sired by an extra-pair male). (b) Proportion of the total number of separating flights in which the female moved away from the male in relation to the start of egg laying (i.e. the day relative to the initiation of the clutch of the focal pair) for pairs with and without extra-pair paternity. Shown are box plots with the median (centre line), 25–75th percentile (limits), minimum and maximum values without outliers (whiskers), and raw data for each day by breeding pair (dots). The grey shaded area indicates the egg laying period, assuming a typical clutch of four eggs. Numbers on top indicate the number of nests with data for each day and category.

Supplementary tables

Table S1. Results of a generalized linear mixed model with the binary variable "together" (yes/no at a given 10-min interval) as the dependent variable and day relative to clutch initiation (i.e. day on which the first egg was laid = 0), clutch initiation date (standardized by subtracting the mean of each year) and time (as sinus and cosinus) as explanatory variables. Data are from the pre-laying period (days -5 to -1). We included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. N_{Nests} = 51 and $N_{\text{Observations}}$ = 18289.

Parameter	**Estimate**	**SE**	**Statistic**	***p***
Intercept	2.825	0.255	11.071	<0.001
Sin(time)	0.318	0.045	7.126	<0.001
Cos(time)	0.185	0.042	4.421	<0.001
Clutch initiation date (linear)	55.914	30.779	1.817	0.07
Clutch initiation date (quadratic)	-100.626	28.965	-3.474	0.001
Day relative to clutch initiation (linear)	130.976	46.028	2.846	0.004
Day relative to clutch initiation (quadratic)	-83.673	5.599	-14.946	<0.001
Random intercept	4.278			
R^2 conditional	0.859			
R^2 marginal	0.074			

Table S2. Results of a generalized linear mixed model with the binary variable "together" (yes/no at a given 10-min interval) as the dependent variable and day relative to clutch initiation (i.e. day on which the first egg was laid = 0), clutch initiation date (standardized by subtracting the mean of each year) and time (as sinus and cosinus) as explanatory variables. Data are from the pre-laying period (days 0 to 3). We included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. $N_{Nests} = 56$ and $N_{Observations} = 19311$.

Parameter	**Estimate**	**SE**	**Statistic**	***p***
Intercept	0.277	0.146	1.903	0.06
Sin(time)	-0.029	0.026	-1.114	0.27
Cos(time)	-0.215	0.025	-8.591	<0.001
Clutch initiation date (linear)	17.239	18.946	0.910	0.36
Clutch initiation date (quadratic)	-74.809	17.163	-4.359	<0.001
Day relative to clutch initiation (linear)	-175.554	16.447	-10.674	<0.001
Day relative to clutch initiation (quadratic)	-5.892	3.043	-1.936	0.05
Random intercept	1.057			
R^2 conditional	0.480			
R^2 marginal	0.303			

Table S3. Results of a generalized linear mixed model with the proportion of time spent together per day as the dependent variable and day relative to clutch initiation (i.e. day on which the first egg was laid = 0), clutch initiation date (standardized by subtracting the mean of each year) and year (2018 and 2019) as explanatory variables. Data are from the pre-laying period (days -5 to -1). We included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. N_{Nests} = 51 (2018: 7, 2019: 44) and $N_{Observations}$ = 189 (2018: 28, 2019:161).

Parameter	**Estimate**	**SE**	**Statistic**	***p***
Intercept	2.390	0.456	5.247	<0.001
Day relative to clutch initiation (linear)	5.147	2.512	2.049	0.04
Day relative to clutch initiation (quadratic)	-4.947	1.222	-4.049	<0.001
Clutch initiation date (linear)	1.499	1.590	0.943	0.35
Clutch initiation date (quadratic)	-6.715	1.723	-3.897	<0.001
Year (2019)	-0.418	0.402	-1.039	0.30
Random intercept	1.701			
R^2 conditional	1.050			
R^2 marginal	0.156			

Table S4. Results of a generalized linear mixed model with the proportion of time spent together per day as the dependent variable and day relative to clutch initiation (i.e. day on which the first egg was laid = 0), clutch initiation date (standardized by subtracting the mean of each year) and year (2018 and 2019) as explanatory variables. Data are from the egg-laying period (days 0 to 3). We included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. N_{Nests} = 56 (2018: 5, 2019: 51) and $N_{Observations}$ = (2018: 17, 2019: 181).

Parameter	**Estimate**	**SE**	**Statistic**	***p***
Intercept	1.315	0.432	3.040	0.002
Day relative to clutch initiation	-0.877	0.079	-11.063	<0.001
Clutch initiation date (linear)	5.372	1.918	2.801	0.005
Clutch initiation date (quadratic)	-5.714	1.651	-3.462	0.001
Year (2019)	0.097	0.446	0.218	0.83
Random intercept	0.548			
R^2 conditional	1.035			
R^2 marginal	0.570			

Table S5. Results of a generalized linear mixed model with the proportion of time spent together per day as the dependent variable and day relative to clutch initiation (i.e. day on which the first egg was laid = 0) and clutch initiation date (standardized by subtracting the mean of each year) as explanatory variables. Data are from the pre-laying period (days -5 to -1). We included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. $N_{Nests} = 51$ and $N_{Observations} = 189$.

Parameter	**Estimate**	**SE**	**Statistic**	***p***
Intercept	1.997	0.164	12.197	<0.001
Day relative to clutch initiation (linear)	4.764	2.145	2.221	0.03
Day relative to clutch initiation (quadratic)	-4.925	1.117	-4.408	<0.001
Clutch initiation date (linear)	1.536	1.450	1.059	0.30
Clutch initiation date (quadratic)	-6.973	1.570	-4.441	<0.001
Random intercept	1.599			
R^2 conditional	1.063			
R^2 marginal	0.161			

Table S6. Results of a generalized linear mixed model with the proportion of time spent together per day as the dependent variable and day relative to clutch initiation (i.e. day on which the first egg was laid = 0) and clutch initiation date (standardized by subtracting the mean of each year) as explanatory variables. Data are from the egg-laying period (days 0 to 3). We included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. N_{Nests} = 56 and $N_{Observations}$ = 198.

Parameter	**Estimate**	**SE**	**Statistic**	***p***
Intercept	1.405	0.130	10.784	<0.001
Day relative to clutch initiation	-0.878	0.079	-11.100	<0.001
Clutch initiation date (linear)	5.348	1.906	2.806	0.005
Clutch initiation date (quadratic)	-5.632	1.612	-3.494	<0.001
Random intercept	0.536			
R^2 conditional	1.035			
R^2 marginal	0.574			

Table S7. Results of a generalized linear mixed model with the proportion of time spent together per day as the dependent variable and day relative to clutch initiation (i.e. day on which the first egg was laid = 0), clutch initiation date (standardized by subtracting the mean of each year) and data type (breeding pair / random pair) in interaction with both as explanatory variables. Data are from the pre-laying period (days -5 to -1). We included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. N_{Nests} = 51 (+178 random pair comparisons) and $N_{Observations}$ = 189 (+239 random pair comparisons).

Parameter	**Estimate**	**SE**	**Statistic**	***p***
Intercept	2.029	0.115	17.664	<0.001
Day relative to clutch initiation (linear)	7.692	2.485	3.095	0.002
Day relative to clutch initiation (quadratic)	-7.819	1.491	-5.243	<0.001
Data type (random pairs)	-4.126	0.150	-27.570	<0.001
Clutch initiation date (linear)	9.207	2.113	4.357	<0.001
Clutch initiation date (quadratic)	-14.289	2.070	-6.901	<0.001
Clutch initiation date (linear):data type (random pairs)	-7.978	2.554	-3.124	0.002
Clutch initiation date (quadratic):data type (random pairs)	13.221	2.428	5.444	<0.001
Random intercept	1.029			
R^2 conditional	1.003			
R^2 marginal	0.832			

Table S8. Results of a generalized linear mixed model with the proportion of time spent together per day as the dependent variable and day relative to clutch initiation (i.e. day on which the first egg was laid = 0), clutch initiation date (standardized by subtracting the mean of each year) and data type (breeding pair / random pair) in interaction with both as explanatory variables. Data are from the egg-laying period (days 0 to 3). We included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. N_{Nests} = 56 (+145 random pair comparisons) and $N_{Observations}$ = 198 (+186 random pair comparisons).

Parameter	**Estimate**	**SE**	**Statistic**	***p***
Intercept	1.352	0.123	11.029	<0.001
Day relative to clutch initiation	-0.859	0.062	-13.910	<0.001
Data type (random pairs)	-3.391	0.172	-19.686	<0.001
Clutch initiation date (linear)	5.189	2.116	2.452	0.014
Clutch initiation date (quadratic)	-7.044	1.950	-3.612	<0.001
Day relative to clutch initiation:data type (random pairs)	0.812	0.088	9.250	<0.001
Clutch initiation date (linear):data type (random pairs)	0.190	2.604	0.073	0.94
Clutch initiation date (quadratic):data type (random pairs)	6.304	2.644	2.384	0.02
Random intercept	0.482			
R^2 conditional	0.949			
R^2 marginal	0.728			

Table S9. Results of a generalized linear mixed model with the proportion of time spent together per day as the dependent variable and day relative to clutch initiation (i.e. day on which the first egg was laid = 0), clutch initiation date (standardized by subtracting the mean of each year) and data type (breeding pair / random pair) in interaction with both as explanatory variables. Data are from after the egg-laying period (days 4 to 10). We included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. N_{Nests} = 38 (+174 random pair comparisons) and $N_{Observations}$ = 180 (+309 random pair comparisons).

Parameter	**Estimate**	**SE**	**Statistic**	***p***
Intercept	-1.297	0.294	-4.412	<0.001
Day relative to clutch initiation	-0.148	0.044	-3.334	0.001
Data type (random pairs)	-0.569	0.376	-1.512	0.13
Clutch initiation date	-0.155	0.031	-4.973	<0.001
Day relative to clutch initiation:data type (random pairs)	0.121	0.055	2.206	0.03
Clutch initiation date:data type (random pairs)	0.158	0.030	5.207	<0.001
Random intercept	0.744			
R^2 conditional	0.351			
R^2 marginal	0.097			

Table S10. Results of a generalized linear mixed model with the binary variable “female move” (yes/no) as the dependent variable and day relative to clutch initiation (i.e. day on which the first egg was laid = 0) and clutch initiation date (standardized by subtracting the mean of each year) as explanatory variables. Data are from the pre-laying period (-5 to -1). We included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. $N_{Nests} = 46$ and $N_{Observations} = 355$.

Parameter	**Estimate**	**SE**	**Statistic**	***p***
Intercept	-0.304	0.287	-1.059	0.29
Day relative to clutch initiation	-0.087	0.085	-1.025	0.31
Clutch initiation date	-0.124	0.048	-2.584	0.01
Random intercept	0.754			
R^2 conditional	0.144			
R^2 marginal	0.055			

Table S11. Results of a generalized linear mixed model with the binary variable “female move” (yes/no) as the dependent variable and day relative to clutch initiation (i.e. day on which the first egg was laid = 0) and clutch initiation date (standardized by subtracting the mean of each year) as explanatory variables. Data are from the egg-laying period (days 0 to 3). We included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. N_{Nests} = 56 and $N_{\mathrm{Observations}}$ = 906.

Parameter	**Estimate**	**SE**	**Statistic**	***p***
Intercept	-0.053	0.164	-0.321	0.75
Day relative to clutch initiation	0.086	0.079	1.097	0.27
Clutch initiation date	0.045	0.028	1.643	0.10
Random intercept	0.710			
R^2 conditional	0.083			
R^2 marginal	0.008			

Table S12. Results of a generalized linear mixed model with the distance moved away when separated (in m) as the dependent variable and with sex, relative clutch initiation day (i.e. day on which the first egg was laid = 0) and day relative to clutch initiation (standardized by subtracting the mean of each year) as explanatory variables. Data are from pre-laying period (days -5 to -1). We included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. $N_{Nests} = 46$ and $N_{Observations} = 355$.

Parameter	**Estimate**	**SE**	**Statistic**	***p***
Intercept	176.404	20.272	8.702	<0.001
Sex (male)	-29.511	16.525	-1.786	0.07
Day relative to clutch initiation	4.284	6.687	0.641	0.52
Clutch initiation date	4.556	3.440	1.325	0.19
Random intercept	14.205			
R^2 conditional	0.197			
R^2 marginal	0.015			

Table S13. Results of a generalized linear mixed model with the distance moved away when separated (in m) as the dependent variable and with sex, relative clutch initiation day (i.e. day on which the first egg was laid = 0) and day relative to clutch initiation (standardized by subtracting the mean of each year) as explanatory variables. Data are from egg-laying period (days 0 to 3). We included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. N_{Nests} = 56 and $N_{Observations}$ = 906.

Parameter	**Estimate**	**SE**	**Statistic**	***p***
Intercept	222.160	28.174	7.885	<0.001
Sex (male)	-21.889	15.438	-1.418	0.16
Day relative to clutch initiation	-4.178	7.743	-0.540	0.59
Clutch initiation date	-2.538	6.249	-0.406	0.69
Random intercept	169.618			
R^2 conditional				
R^2 marginal	0.004			

Table S14. Results of a generalized linear mixed model with the proportion of time spent at the nest per dayas the dependent variable and relative clutch initiation day (i.e. day on which the first egg was laid = 0) and day relative to clutch initiation (standardized by subtracting the mean of each year) as explanatory variables. Data are from the egg-laying period (days 0 to 3). We included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. N_{Nests} = 56 and $N_{\text{Observations}}$ = 198.

Parameter	**Estimate**	**SE**	**Statistic**	***p***
Intercept	-1.314	0.097	-13.562	<0.001
Day relative to clutch initiation	0.553	0.071	7.753	<0.001
Clutch initiation date	0.039	0.023	1.723	0.09
Random intercept	0.461			
R^2 conditional	0.915			
R^2 marginal	0.301			

Table S15. Results of a generalized linear mixed model with the proportion of time spent at the nest per day as the dependent variable and relative clutch initiation day (i.e. day on which the first egg was laid = 0) and day relative to clutch initiation (standardized by subtracting the mean of each year) as explanatory variables. Data are from the egg-laying period (days 0 to 3). We included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. N_{Nests} = 56 and $N_{\text{Observations}}$ = 198.

Parameter	**Estimate**	**SE**	**Statistic**	***p***
Intercept	-1.594	0.101	-15.761	<0.001
Day relative to clutch initiation	-0.251	0.068	-3.671	<0.001
Clutch initiation date (linear)	4.685	1.439	3.255	0.001
Clutch initiation date (quadratic)	-3.947	1.316	-2.999	0.003
Random intercept	0.436			
R^2 conditional	0.785			
R^2 marginal	0.227			

Table S16. Results of a generalized linear mixed model with the proportion of time spent together per day as the dependent variable and relative clutch initiation day (i.e. day on which the first egg was laid = 0), day relative to clutch initiation (standardized by subtracting the mean of each year) and extra-pair paternity (whether at least one egg in the clutch was sired by an extra-pair male, yes/no; EPP) as explanatory variables. Data are from the pre-laying period (days -5 to -1). We included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. N_{Nests} = 49 (with EPP: 5, without EPP: 44) and $N_{Observations}$ = 183 (with EPP: 18, without EPP: 165).

Parameter	**Estimate**	**SE**	**Statistic**	***p***
Intercept	2.019	0.179	11.280	<0.001
Day relative to clutch initiation (linear)	4.847	2.303	2.104	0.04
Day relative to clutch initiation (quadratic)	-4.830	1.143	-4.224	<0.001
Clutch initiation date (linear)	1.463	1.641	0.892	0.37
Clutch initiation date (quadratic)	-7.099	1.737	-4.088	<0.001
EPP (yes)	-0.208	0.409	-0.508	0.61
Random intercept	1.704			
R^2 conditional	1.055			
R^2 marginal	0.143			

Table S17. Results of a generalized linear mixed model with the proportion of time spent together per day as the dependent variable and relative clutch initiation day (i.e. day on which the first egg was laid = 0), day relative to clutch initiation (standardized by subtracting the mean of each year) and extra-pair paternity (whether at least one egg in the clutch was sired by an extra-pair male, yes/no; EPP) as explanatory variables. Data are from the fertile egg-laying period (0 to 2). We included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. N_{Nests} = 54 (with EPP: 6, without EPP: 48) and $N_{Observations}$ = 147 (with EPP: 17, without EPP: 130).

Parameter	**Estimate**	**SE**	**Statistic**	***p***
Intercept	1.425	0.140	10.150	<0.001
Day relative to clutch initiation	-0.935	0.090	-10.414	<0.001
Clutch initiation date (linear)	4.661	1.650	2.824	0.005
Clutch initiation date (quadratic)	-5.518	1.528	-3.612	<0.001
EPP (yes)	0.505	0.413	1.225	0.22
Random intercept	0.619			
R^2 conditional	1.035			
R^2 marginal	0.530			

Table S18. Results of a generalized linear mixed model with the binary variable "female move" (yes/no at a given 10-min interval) as the dependent variable and relative clutch initiation day (i.e. day on which the first egg was laid = 0), day relative to clutch initiation (standardized by subtracting the mean of each year) and extra-pair paternity (whether at least one egg in the clutch was sired by an extra-pair male, yes/no; EPP) as explanatory variables. Data are from the pre-laying period (-5 to -1). We included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. N_{Nests} = 46 (with EPP: 5, without EPP: 39) and $N_{Observations}$ = 355 (with EPP: 56, without EPP: 290).

Parameter	**Estimate**	**SE**	**Statistic**	***p***
Intercept	-0.309	0.302	-1.021	0.31
Day relative to clutch initiation	-0.056	0.088	-0.633	0.53
Clutch initiation date	-0.120	0.051	-2.367	0.02
EPP (yes)	0.692	0.455	1.521	0.13
Random intercept	0.804			
R^2 conditional	0.167			
R^2 marginal	0.088			

Table S19. Results of a generalized linear mixed model with the binary variable "female move" (yes/no at a given 10-min interval) as the dependent variable and relative clutch initiation day (i.e. day on which the first egg was laid = 0), day relative to clutch initiation (standardized by subtracting the mean of each year) and extra-pair paternity (whether at least one egg in the clutch was sired by an extra-pair male, yes/no; EPP) as explanatory variables. Data are from the fertile egg-laying period (0 to 2). We included nest ID as a random effect allowing for random slopes over the day relative to clutch initiation. N_{Nests} = 53 (with EPP: 6, without EPP: 47) and $N_{Observations}$ = 715 (with EPP: 91, without EPP: 624).

Parameter	**Estimate**	**SE**	**Statistic**	***p***
Intercept	0.070	0.184	0.381	0.70
Day relative to clutch initiation	0.002	0.112	0.020	0.98
Clutch initiation date	0.023	0.030	0.766	0.44
EPP (yes)	0.113	0.313	0.360	0.72
Random intercept	0.751			
R^2 conditional	0.080			
R^2 marginal	0.002			

Chapter 3

Wind conditions influence breeding season movements in a nomadic polygynous shorebird

Johannes Krietsch, Mihai Valcu and Bart Kempenaers

Nomadism is a behaviour where individuals respond to environmental variability with movements that seem unpredictable in timing and direction. In contrast to migration, the mechanisms underlying nomadic movements remain largely unknown. Here, we focus on a form of apparent nomadism in a polygynous shorebird, the pectoral sandpiper (*Calidris melanotos*). Local mating opportunities are unpredictable and most males sampled multiple sites across a considerable part of their breeding range. We test the hypothesis that individuals decided which part of the breeding range to sample in a given season based on the prevailing wind conditions. Using movement data from 80 males in combination with wind data from a global reanalysis model, we show that male pectoral sandpipers flew with wind support more often than expected by chance. Stronger wind support led to increased ground speed and was associated with a longer flight range. Long detours (loop-like flights) can be explained by individuals flying initially with the wind. Individuals did not fly westwards into the Russian Arctic without wind support, but occasionally flew eastwards into the North American Arctic against strong headwinds. Wind support might be less important for individuals flying eastwards, because their autumn migration journey will be shorter. Our study suggests that individuals of a species with low site fidelity choose their breeding site opportunistically based on the prevailing wind conditions.

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Wind conditions influence breeding season movements in a nomadic polygynous shorebird

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Nomadism is a behaviour where individuals respond to environmental variability with movements that seem unpredictable in timing and direction. In contrast to migration, the mechanisms underlying nomadic movements remain largely unknown. Here, we focus on a form of apparent nomadism in a polygynous shorebird, the pectoral sandpiper (*Calidris melanotos*). Local mating opportunities are unpredictable and most males sampled multiple sites across a considerable part of their breeding range. We test the hypothesis that individuals decided which part of the breeding range to sample in a given season based on the prevailing wind conditions. Using movement data from 80 males in combination with wind data from a global reanalysis model, we show that male pectoral sandpipers flew with wind support more often than expected by chance. Stronger wind support led to increased ground speed and was associated with a longer flight range. Long detours (loop-like flights) can be explained by individuals flying initially with the wind. Individuals did not fly westwards into the Russian Arctic without wind support, but occasionally flew eastwards into the North American Arctic against strong headwinds. Wind support might be less important for individuals flying eastwards, because their autumn migration journey will be shorter. Our study suggests that individuals of a species with low site fidelity choose their breeding site opportunistically based on the prevailing wind conditions.

1. Introduction

Flying or swimming animals move within a medium which is in motion itself. This means that the animal's trajectory is the result of their own speed and heading, and the speed and direction of the flow of the medium [1]. In birds, a growing body of literature describes the substantial effects of wind on flight, mainly within the framework of 'optimal migration' [2–4]. Migratory birds can optimize their flight efficiency (i.e. time in flight, flight range and energy expenditure) in relation to wind by (i) selecting a departure time with the most favourable winds [3,5,6], (ii) choosing a flight altitude with the most favourable winds [7–9], (iii) choosing an optimal flight speed and mode (i.e. flapping, gliding or soaring) [10–13], and (iv) adjusting their route to use the most favourable winds [14–17]. Whether and how individuals can use the wind during flights depends on species-specific differences in life history and ecology that allow different degrees of variability regarding timing of movement and space use [18–23]. Most migratory birds show high philopatry, returning to the same breeding, stopover and wintering sites each year [24]. Some species also have highly repeatable routes, but can be flexible in their timing of migration (e.g. [16]), while other species adjust their route based on wind conditions, despite being constrained in time [18–20]. For individuals flying towards a specific goal, the use of optimal wind conditions might be constrained or overridden by other factors. For example, individuals might face trade-offs between waiting for favourable winds and optimal timing [2,21,22], or selection might favour individuals that avoid ecological barriers or cross them on the shortest route, independently of the wind conditions. Ultimately, these factors may define whether individuals benefit from being flexible or consistent with respect to timing and route (e.g. [18]), and consequently determine whether consistency or plasticity in movements are favoured by selection.

Here, we consider wind use during a recently discovered form of nomadic movement in a species that shows extremely low site fidelity, the pectoral sandpiper (*Calidris melanotos*). Pectoral sandpipers are polygynous and males compete for access to fertile females, but provide no parental care [25,26]. In spring, individuals migrate from their wintering grounds in the Southern Hemisphere (mostly South America) to the Arctic. However, most males do not stay at one breeding site. Presumably in response to variation in local mating opportunities, males sample several potential breeding sites during the four to six weeks lasting breeding season, often throughout a considerable part of the species's breeding range, covering distances of up to 13 000 km [27]. Individuals can move between successive sites in any direction, at any time and over variable distances [27,28]. The low local return rates of females (less than 1% [29]) and large between-year variation in snow accumulation and timing of snowmelt in the Arctic [30] result in locally unpredictable mating opportunities [27] and presumably in substantial spatio-temporal variation in breeding opportunities throughout the species's breeding range.

Given that local mating opportunities are unpredictable [27], that local competition is intense and presumably energetically costly [31] and that time is an important resource (short breeding season in the Arctic), males may use wind support to move to the next site quickly and with the least energy expenditure. Because the quality of a distant site is unpredictable, the costs associated with reaching a particular site should be important for individuals making decisions about where to go next. Wind patterns over the Arctic Ocean are highly variable between years and within a season [32]. Thus, the aim of our study is to investigate (i) whether individual pectoral sandpipers use the local wind conditions to decide in which part of the breeding range they will sample potential breeding sites and (ii) how the wind conditions en route influence their flights.

We used movement data from 80 males that departed from a breeding site in the centre of their breeding range. Males flew either west into the Russian Arctic, or east into the North American Arctic, but the proportion going west differed between years [27]. Using wind data from a global reanalysis model [33], we first assessed how wind conditions influenced the males' flight. We characterized the wind conditions connected to the flights and estimated the most likely flight altitude. We then analysed the effect of wind support on ground and air speed, predicting that increasing wind support resulted in both faster ground speeds (i.e. reduced flight time) and lower air speeds (i.e. reduced flight costs) [34,35]. Second, we analysed whether the direction of male movements was associated with wind conditions within and between years. We examined whether the local wind direction and speed influenced the males' initial flight direction. We then asked if the initial flight direction predicted where individuals settled next. We also tested whether individuals timed their departure to make use of optimal wind support, assuming that their final destination was their goal. Finally, we compared the wind support on the actual track with that on the shortest route to the destination to assess whether the use of wind support can explain the observed large detours (loop flights).

2. Material and methods

(a) Tracking data

In both 2012 and 2014, 60 adult male pectoral sandpipers were caught and equipped with 5 g Solar Argos PTT-100 (Microwave Telemetry Inc.) satellite transmitters near Utqiaġvik (Barrow), Alaska (71°18′ N, 156°44′ W) between 25 May and 7 June. All transmitters had a continuous duty cycle and we obtained on average 2.8 positions per hour for the flights analysed here. Raw Argos data were filtered and a continuous time-correlated random walk model was used to predict maximum-likelihood locations every 15 min. For further details about tag attachment and data processing, see [27]; the complete dataset is available at http://dx.doi.org/10.17605/osf.io/vx2mk.

For this study, we selected a subset of tracks with the following criteria: (i) departure date before 8 July (excluding movements after the latest clutch initiation date, based on [36]), (ii) departure location less than 250 km from Utqiaġvik, (iii) departure track over the Chukchi or Beaufort Sea (excluding four over-land tracks), and (iv) track length greater than 500 km (figure 1). Because males first flew a considerable distance over sea (ice), we can exclude the possibility that they were assessing conditions on land (e.g. snow cover) to decide where to settle next. In total, 85 tracks (2012: $n = 49$; 2014: $n = 36$) from 80 individuals fulfilled these criteria and were used for further analyses. In both years, the area covered by the flight paths was almost entirely covered with sea ice (electronic supplementary material, figure S1). Thus, we can exclude that variation in ice cover played a role in decisions about where to go.

Five males are included twice, because they flew two tracks within a season that fitted our selection criteria. Four of them made a loop flight (see below), followed by a directional flight, while one first flew east, then west. A sixth male made a loop flight, followed by a directional flight that would have fitted the selection criteria, except that the departure location was greater than 250 km from Utqiaġvik.

(b) Track description

For each track, we defined the following variables. (i) Departure time: time of the first position over the ocean. Males mainly left during the 'night'. Thus, for analyses that included departure day, we centred the days around midnight by subtracting 12 h from each day, such that all birds that left in one night were counted as belonging to one departure 'day'. (ii) Arrival time: time of the last position over the ocean. We excluded positions over land until the next residency area (identified as spatial clusters of points; for details see [27]) was reached, because the environmental cues might change in comparison to those while flying over the ocean. In most cases, the distance from the coast to the residency area was small relative to total track length (median: 73 km, range: 1–1147 km). (iii) Flight time: the period between departure and arrival; birds did not rest on the sea ice [27]. (iv) Track length: the sum of the distances between all consecutive positions between departure and arrival. (v) Track detour: the difference between track length and the shortest distance between departure and arrival location. (vi) Percentage detour: track detour divided by track length. (vii) Track straightness: track length divided by the shortest distance between the departure and arrival location [37]. A straightness of 1 indicates a direct flight between two points.

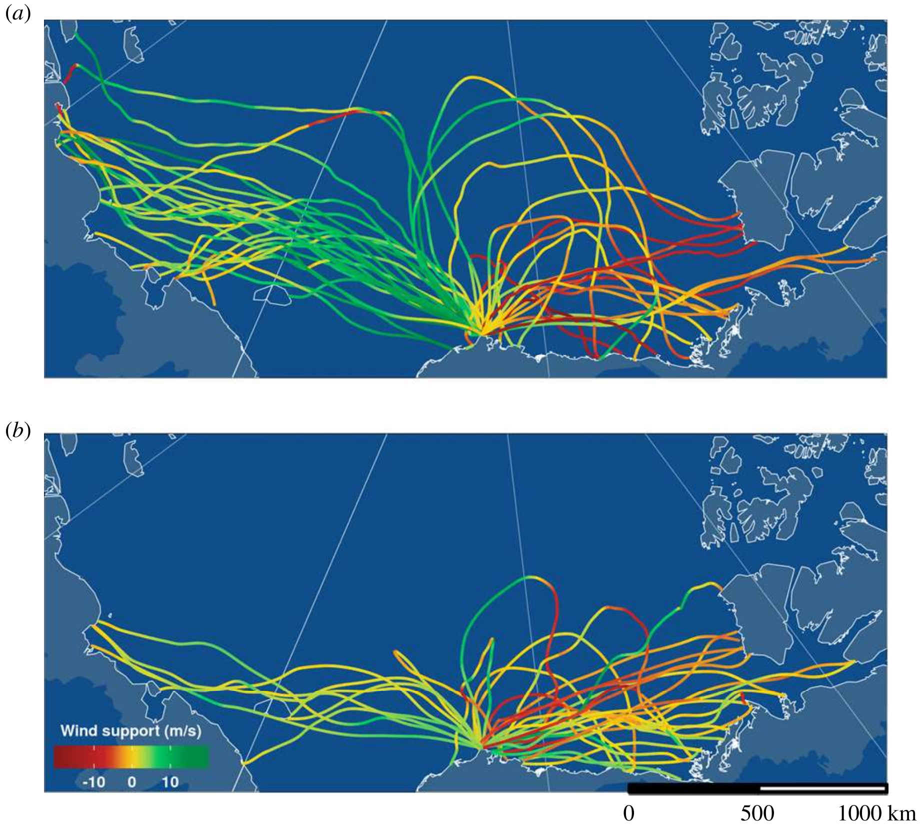

Figure 1. Tracks of male pectoral sandpipers that left Utqiaġvik, Alaska (lower centre of the map; capture site) during the breeding season between 30 May and 23 June in 2012 (*a*; $n = 49$) and 2014 (*b*; $n = 36$). Track colour indicates wind support, i.e. the length of the wind vector in the direction of the bird's movement (ground vector), calculated using wind data at approximately 750 m altitude (see Material and methods). Tailwinds are shown in green, headwinds in red. The grey-blue area indicates suitable breeding habitat within the known breeding range of the species [27]. Map projection: polar Lambert azimuthal equal area with longitude origin 156.65° W (Utqiaġvik) from Natural Earth (http://www.naturalearthdata.com). For a movie of these flights and for individual tracks see electronic supplementary material (movie available at https://www.youtube.com/watch?v=A-Q5J1wRBUA&feature=youtu.be). (Online version in colour.)

(c) Wind data

We used wind data from the ECMWF ERA-Interim reanalysis [33] with a spatial resolution of approximately 80 km/0.75° and a temporal resolution of 6 h. We extracted the u- (west–east) and v- (south–north) wind components for six altitudes: 10 m above ground and at the pressure levels 1000, 925, 850, 775 and 700 hPa, roughly corresponding to 100, 750, 1500, 2250 and 3000 m.a.s.l. (metres above-sea level). The actual flight altitudes are unknown, but we chose these altitudes because radar data from the region indicate that shorebirds (including pectoral sandpipers) mainly used altitudes between 0 and 3000 m.a.s.l. [38]. Data were resampled with a bicubic interpolation to a spatial resolution of 10 km. To test whether our results are sensitive to the time scale of the wind data, we shifted all tracks ±6 h and calculated Pearson product–moment correlation coefficients comparing the mean wind support for each altitude (see below). Shifted and observed data correlated strongly (all $r > 0.98$, $n = 85$). Thus, the temporal resolution of the wind data is adequate. To assess the effect of wind on departures at a finer temporal scale, we used local wind data from the weather station in Utqiaġvik (https://www.esrl.noaa.gov). These data contain hourly averages of wind speed and direction at ground level.

(d) Wind conditions in the area of the flights

We defined the area used by the males in our dataset during their flights over the ocean as a convex hull covering all tracks and excluding all land surface. Based on the known breeding range and suitable habitat of the species [27], we defined the corresponding area over land the birds could have used to move to the same site (with the exception of crossing the Bering Strait). For both areas ('over ocean' and 'over land'), we calculated daily mean wind speeds and directions for the period of actual departures.

(e) Wind support, relative wind support and crosswind

For each individual, we calculated ground speed and direction of movement (0° as north) between subsequent points on its track. Using wind data (at the six altitudes) closest in space and time to each position, we calculated wind support as the length of the wind vector in the direction of the bird's flight (ground vector) as described in [11]. Positive values represent tailwinds, negative values headwinds.

To quantify how birds used the wind independently of wind speed, we calculated 'relative wind support' as wind support divided by wind speed. Values range between −1, for birds that fly in the exact opposite direction to the wind (headwind), and +1, for birds that fly exactly in the direction of the wind (tailwind).

We calculated the crosswind component as the length of the wind vector perpendicular to the ground vector, regardless of the side it came from [11].

(f) Altitude with maximal wind support and used flight altitude

Birds can vary their flight altitude to optimally use the wind conditions along the route [9,39]. We calculated the altitude at which each male would have experienced the highest mean wind support, as follows. We computed average mean wind support for 1 h flight intervals for all six altitudes (i.e. allowing a change to any altitude each hour). The altitude with maximal wind support (hereafter 'maxWs' altitude) was then defined as the altitude with highest mean wind support for each 1 h interval.

To estimate the altitude at which males were most likely to have flown, we fitted linear mixed-effect models with the birds' ground speed as the dependent variable and with wind support and cross wind at a given altitude as the explanatory variables. Thus, we fitted seven models: one for each fixed altitude and one for the maxWs altitude as defined above. We included track ID as random effect and used a moving-average correlation structure to control for temporal autocorrelation. We identified the altitude that best explained the observed ground speeds based on a maximum-likelihood approach using Akaike's information criterion (AIC), selecting the model with the lowest AIC. We then compared the mean wind support during the first 50 km at 10 m with that at the most likely flight altitude and assessed whether wind support at both altitudes was correlated (i.e. whether individuals could predict the wind conditions at flight altitude based on the wind close to ground level).

(g) Wind and flight performance

To assess the influence of the wind on ground and air speed, we used the model as described above and a similar model with air speed (calculated as ground speed minus wind support) as the dependent variable.

For nomadic movements, we expect that individuals will opportunistically cover longer distances over sea if they experience stronger wind support, assuming everything else (e.g. condition) is equal. This implies that the amount of wind support will influence in which part of the breeding range a male pectoral sandpiper will arrive to search for suitable breeding habitat and potentially establish a territory. To test whether males covered longer distances over sea before reaching land with higher wind support, we fitted a linear model with track length as the dependent variable and with mean wind support during the first half of the track as the explanatory variable.

(h) Wind and direction and timing of departure

To test whether local wind conditions predict the initial flight direction and whether the initial flight direction in turn predicts where the birds reach land (i.e. suitable breeding habitat), we fitted two linear mixed-effect models. In the first model, we used the initial flight direction (mean of first 50 km) as the dependent variable, and wind (i.e. interaction between mean wind direction and wind speed during the first 50 km of flight) as the explanatory variable. In the second model, the final flight direction (i.e. the direction between departure and arrival location) was the dependent variable and the initial flight direction the explanatory variable. In both models, we included date (night in a given year) as a random effect.

Second, we asked whether the expected wind support predicted how many males flew either to the Russian or to the North American Arctic on a given day. Using all observed, directed flights, either to the Russian or to the North American Arctic ($n = 78$), we computed wind support at the mean departure time (22.30 Alaska Daylight Time, AKDT) for every day during which departures took place (27 May–23 June). For each departure night, we then calculated the difference in wind support an individual would have experienced when flying east or west (delta wind support: $\Delta_{\mathrm{W-E}}$) and linked this to the actual departures in each direction. We then tested whether the observed wind support was higher than the wind support expected by chance. Using all flights ($n = 85$), we simulated 10 000 random departures for each track within the first and last departure date of each season and compared the mean wind support of the simulated flights with the actual observed mean wind support.

Even if males flew with wind support, this does not necessarily imply that they simply went 'with the flow'. The same pattern could arise if individuals waited for favourable wind conditions to depart to their targeted area. Thus, we tested whether males shifted their departure time to match favourable wind conditions, both within a given day (night) and between days. We calculated wind support at the departure location assuming a direct flight to their actual destination (i.e. assuming that each individual had that location as their goal) at the actual departure times ±12 h in hourly steps (within-day decisions, based on local wind data at ground level) and at the actual departure date ±5 days in daily steps (between-day decisions, based on the reanalysis model at six fixed altitudes). If males indeed timed their departure in response to favourable winds, we expect worse wind conditions (i.e. less wind support) to fly to the same destination in the hours or days before or after the actual departure to that destination.

(i) Comparing wind support for the shortest route and the actual track

For each altitude, we compared the mean wind support on the actual route with the mean wind support the bird would have experienced had it taken the shortest route. To estimate wind support on the shortest path, we simulated for each individual its movement from the departure to the arrival location, assuming a constant heading towards the latter. We then estimated movement speed using the local wind support and crosswind and the ground speed predicted for such wind conditions. The latter was calculated based on the linear mixed-effect model with observed ground speed as the dependent variable (see §2(f)). To match the temporal pattern of the observed tracks, we assumed that the bird flew 15 min with this predicted ground speed, resulting in the next position on the track. If at any position along the shortest track the predicted ground speed was negative, we conservatively assumed that the bird did not move in this 15 min interval (instead of going backwards).

(j) Data analysis

We performed all analyses with R [40]. Spatial data were transformed to a polar Lambert azimuthal equal-area projection with longitude origin at Utqiaġvik (156.65° W).

We created the R package 'windR' (available at https://github.com/mpio-be/windR), which provides a set of tools to connect flight tracks with wind data and to calculate the wind support and crosswinds. Additionally, the package provides a set of graphical methods to create particle flow animations.

We fitted linear mixed-effect models with the package 'lme4' [41] and 'nlme' [42]. For multiple predictor models, we used the package 'multcomp' [43] to compute adjusted p-values from the corresponding t or z multivariate distribution to account for the correlations between the parameter estimates.

3. Results

(a) Flight description

Males departed between 30 May and 17 June (mean ± s.d.: 7 June ± 3 days, excluding one outlier on 23 June), with a mean local departure time of 22.30 (±3.3 h; range: 13.20–08.20 AKDT). Males flew 7–55 h non-stop over sea (mean ± s.d.: 24 ± 8 h), covering 584–2609 km (mean ± s.d.: 1360 ± 456 km). Thus, the average ground speed was $16 \pm 3\ \mathrm{m\ s^{-1}}$

(58 ± 11 km h^{-1}); the fastest male reached an average speed of 22 m s^{-1} (78 km h^{-1}).

The percentage detour was overall low (median: 7%), but ranged between less than 1% and 91%. We classified flights into ‘directed flights’ (92%, median straightness: 1.1, range: 1.0–2.3) and ‘loop flights’ (8%, median straightness: 7.8, range: 5.1–11.1; figure 1; electronic supplementary material, tracks). Directed flights either went east (i.e. visited areas in the North American Arctic, 44% in 2012 and 76% in 2014) or west (i.e. to the Russian Arctic, 56% in 2012 and 24% in 2014).

(b) Wind conditions and wind support during the flights

Wind conditions varied between years (electronic supplementary material, figure S2). In 2012, strong winds (6–10 m s^{-1}) blowing in northwesterly direction dominated, whereas in 2014, wind direction was more variable with lower speeds. Wind speeds over the ocean were on average 1.2 m s^{-1} higher than over the land (electronic supplementary material, figure S2).

Wind support did not differ much between the six altitudes (maximum difference less than 1.5 m s^{-1}; electronic supplementary material, figure S3). However, if males would have flown at the altitude with maximum wind support during each hour along the track (see Material and methods), they would have experienced 2.2–3.4 m s^{-1} higher wind support in comparison to flying at a fixed altitude with 0.6–1.8 m s^{-1} wind support. The altitude with the highest wind support varied within and between tracks. For all tracks combined, the mean percentage of the track with maximal wind support at a given altitude was 7% at 10 m, 27% at 100 m, 18% at 750 m, 4% at 1500 m, 4% at 2250 m and 39% at 3000 m.

The estimated wind support and crosswind at 750 m fitted best to the observed ground speeds (electronic supplementary material, table S1) and was therefore probably the most used altitude. Thus, all further results are from analyses assuming flights at 750 m (conclusions remain the same for the other six altitudes, details not shown). During the first 50 km of each flight, the mean wind support at 10 m and at 750 m correlated strongly (Pearson’s $r = 0.92$, $n = 85$, $p < 0.001$).

(c) Influence of wind conditions on flight performance

With increasing wind support, ground speed increased, while estimated airspeed (the birds’ flying effort) decreased (electronic supplementary material, table S2). However, the effect on ground and estimated air speed decreased with increasing crosswinds (electronic supplementary material, table S2).

Wind support during the first half of the track predicted total track length (electronic supplementary material, table S3), after controlling for direction (i.e. east, west or loop flight). The latter is necessary because land is typically reached earlier when going east. Each additional 1 m s^{-1} wind support increased the overall track on average by 22 km.

(d) Influence of wind conditions on the trajectory and on the direction and timing of departure

Overall, 70 out of 85 males (82%) left with a positive wind support at departure (mean wind support during the first 50 km of the track: 4.9 m s^{-1}, range: 0.1–14.9 m s^{-1}; figure 1).

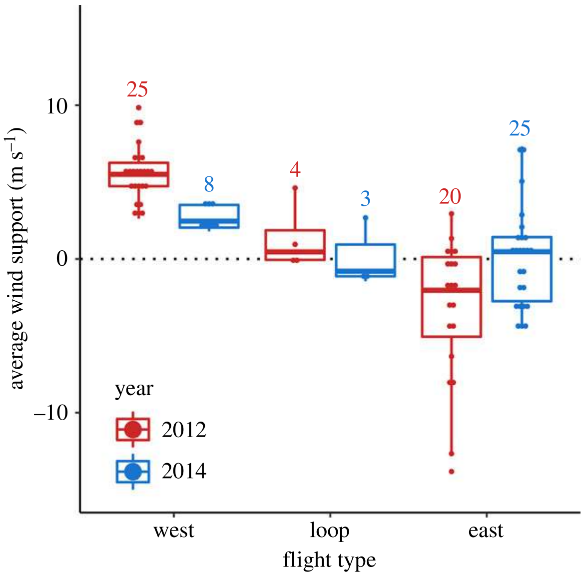

Figure 2. Average wind support (calculated based on wind data at approximately 750 m altitude; see Material and methods) for the entire flight in relation to flight type and year. Loop flights are defined as tracks with a low straightness (median = 7.8). Westward and eastward flights are defined as tracks directed toward their respective destination with high straightness (see Material and methods). Shown are box-plots with median (centre line), 25–75th percentile (limits), minimum and maximum values without outliers (whiskers), and outliers (dots). The number above the box indicates the number of tracks in each category. (Online version in colour.)

The other 18% of males left with headwinds ranging from −0.2 to −10.9 m s^{-1} (mean: −3.3 m s^{-1}; figure 1). All males that flew west experienced on average positive wind support, whereas males that went east more often faced headwinds (figures 1 and 2; electronic supplementary material, figure S4 and movie available at https://www.youtube.com/watch?v=A-Q5J1wRBUA&feature=youtu.be). The observed mean wind support during the first 50 km of the track was significantly higher than the mean wind support of a random departure within the period of departures in the same season in 97% of cases (based on 10 000 simulations; mean difference: 2.5 m s^{-1}, range: 0.5–4.2 m s^{-1}). This was also true when the mean wind support during the entire track was considered, yet the difference between the observed and randomized tracks was smaller (mean difference: 1.7 m s^{-1}, range: 0.2–3.1 m s^{-1}; significant for 94% of 10 000 simulations).

The initial flight direction was related to wind direction, with the effect becoming stronger with increasing wind speed (electronic supplementary material, figure S5a and table S4), and was a good predictor of where males became resident next (electronic supplementary material, figure S5b and table S5). As a result, wind conditions at the departure location influenced the number of males that became resident in the Russian versus the North American Arctic (figure 3; electronic supplementary material, table S6).

Whereas initially most birds left with the wind, wind support gradually decreased along the track to average values around zero (electronic supplementary material, figure S6a and table S7). In general, the relationship between wind direction and flight direction (i.e. relative wind support) increased with wind speed (electronic supplementary material, figure S6b).

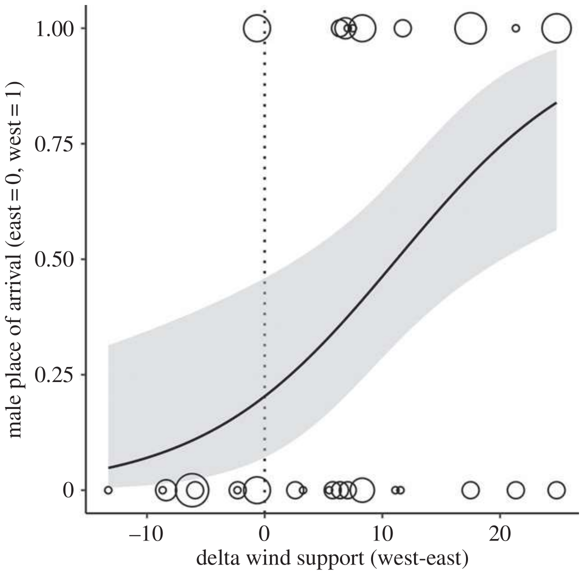

Figure 3. Site of arrival in relation to the average difference in wind support for going west (i.e. into the Russian Arctic, $n = 33$) versus east (i.e. into the North American Arctic, $n = 45$) during the first 50 km. Delta wind support was calculated by subtracting the mean wind support for all tracks that went east from the mean wind support for all tracks that went west. Dot size reflects the number of individuals that left during a given night in the same direction ($n = 1$–8). Shown are model estimates (lines) and 95% confidence intervals (grey areas). See electronic supplementary material table S6 for model descriptions.

The difference in wind patterns between years (see above) coincided with differences in the proportion of individuals that flew to the Russian and North American Arctic. In 2012, 56% of the males went west, benefiting from strong tailwinds (5.4 ± 4.7 m s^{-1}), while males that went east on average faced headwinds (–3.0 ± 5.5 m s^{-1}; figures 1 and 3). In 2014, when winds were generally weaker, only 24% of the males went west and males leaving in both directions on average departed with tailwinds (west: 2.7 ± 1.6 m s^{-1}; east: –0.3 ± 3.7 m s^{-1}; figures 1 and 3; electronic supplementary material, table S8).

Of all males, 35% started a long over-sea flight within 3 days after capture, 57% within 5 days and the remaining males left 6–24 days after capture. Consequently, most males could have adjusted their departure date to use favourable winds to fly towards a hypothetical destination. However, we found no evidence that they would have experienced worse (or better) wind support when shifting their departure by 1–5 nights or by 1–12 h within a night (electronic supplementary material, table S9).

(e) Wind support on the shortest versus the actual route

Wind support on the actual track was higher than on the shortest route for males going east (mean difference between actual and shortest: 2.1 m s^{-1} ± 0.8 s.e., $p = 0.019$; electronic supplementary material, table S10) and non-significantly smaller for males going west (mean: –1.5 m s^{-1} ± 0.9 s.e., $p = 0.20$). However, males that would have taken the direct route would on average have spent less time flying (assuming they flew with a ground speed predicted for the local wind support and crosswind; see Material and methods), independent of whether they went east (mean difference: 2.57 h ± 1.02 s.e., $p = 0.034$) or west (mean difference: 0.53 h ± 1.22 s.e., $p = 0.96$; electronic supplementary material, table S10).

For loop flights, wind support on the actual track was much higher than it would have been on the direct route (mean difference between actual and shortest: 3.5 m s^{-1} ± 1.3 s.e., $p = 0.020$). Nevertheless, males spent much more time flying than if they would have taken the shortest route (mean difference: 23.28 h ± 2.43 s.e., $p < 0.001$). Visual inspection shows that four individuals (114 272, 114 273, 114 279 and 114 307, see electronic supplementary material, tracks) flew a major detour before ending up in the Canadian Arctic.

4. Discussion

Little is known about the mechanisms underlying nomadic movements [44]. In this study, we focused on nomadic movements by a polygynous shorebird during the breeding season [27]. Our results suggest that the direction in which pectoral sandpipers flew to sample other potential breeding sites is influenced by the strength and direction of the winds at the departure location. We show that most males flew with wind support, especially those flying to the Russian Arctic. We found no evidence that males waited for optimal wind to leave to a specific target area. Wind conditions influenced both in which direction males left and how far they flew and thus ultimately in which region they arrived and potentially reproduced. Between-season variation in wind conditions influenced the proportion of males that sampled sites in the Russian versus the North American Arctic. For six males with two recorded long flights within the same season, three left twice in the same direction, while the other three flew in opposite directions (electronic supplementary material, tracks). In conclusion, most male pectoral sandpipers seem to opportunistically fly in the direction that provided wind support. As expected, higher wind support led to higher ground speeds at lower air speeds and to longer total flight distances.

(a) Flight characteristics and wind support

The choice of the most favourable altitude is an important part of an individual's adaptive exploitation of winds [2,3,9]. Our results suggest that males predominantly flew at approximately 750 m.a.s.l., which falls within the range estimated by radar within the Beringia region [38]. We found no evidence that birds adjusted their altitude during flight to obtain maximal wind support (electronic supplementary material, table S1). However, changes in flight altitude did not strongly change wind support (electronic supplementary material, figure S3). Only further studies using transmitters that measure atmospheric pressure will be able to reveal the actual flight altitude.

The majority of males left our study site by flying over the ice-covered sea, rather than over the land where they could have rested or assessed local breeding conditions continuously. Flying over the sea was typically a shorter route to their destination. Higher wind speeds over the sea would have benefited individuals that flew with tailwind, but males facing headwinds would have done better by flying over the land.

(b) Influence of wind conditions on flight performance

Males adjusted their air speed depending on experienced wind support, flying faster with headwinds and slower with tailwinds (electronic supplementary material, table S2). This relationship is expected as a way to minimize energy expenditure [34,35] and generally described for birds [45]. Ground speeds increased with tailwinds and decreased with headwinds, but these effects were weakened by cross winds, presumably because individuals changed their heading to compensate for wind drift. Overall, our results suggest that wind support likely had a substantial influence on the energy expenditure of males. With tailwinds, males flew slower, but moved faster and further than males flying with headwinds (electronic supplementary material, tables S2 and S3). On one occasion, a male turned around, presumably to avoid an approaching storm with strong headwinds (see electronic supplementary material, movie available at https://www.youtube.com/watch?v=A-Q5J1wRBUA&feature=youtu.be, 10 June 2014).

(c) Influence of wind conditions on breeding site sampling

The males' initial flight direction was influenced by wind direction and speed, such that most birds initially left with tailwinds, especially when wind speeds were high (figures 2*a*,*b* and 3). The wind conditions at ground level (10 m) correlated strongly with those at the most likely flight altitude (750 m). Males could thus use the wind conditions on the ground to predict the wind conditions they would experience during their flight. For all flights, the observed wind support was higher than the wind support individuals would have experienced had they left on a random day within the same period. However, we found no evidence that males waited for favourable wind conditions to depart (electronic supplementary material, table S9). Given the short breeding season and the unpredictable conditions (opportunities to mate) at other potential breeding sites, waiting for optimal winds to reach a particular goal may reduce the probability to reproduce in a given year.

Along the track, the relationship between wind direction and track direction weakened. Thus, males initially moved 'with the flow', but most kept a rather constant flight direction, which usually resulted in a decrease of wind support along the track. Some individuals initially flew in a northerly direction, before turning east or west (figure 1). These movements mirror the strategy of 'adaptive drift' [46], where birds can gain time and energy by allowing an initial ground speed increase due to drift followed by displacement compensation toward the end of the trip. Given the shape of the breeding range this compensation was necessary to reach land (figure 1). Consequently, most males did not simply fly 'with the flow' for the entire track over sea, but may have aimed towards a broad goal. Once the males reached land, the majority settled after a short distance, but a few continued flying longer distances. The factors that determine the local site selection remain unknown, but direct cues about the suitability of a potential breeding site such as snow cover or presence of conspecifics may be more important than wind conditions.

Several males faced moderate to strong headwinds directly after departure and over their entire journey (figures 1 and 3). This suggests that some individuals may have a preferred direction. Only 2% of males returned to our study area between years and these were typically males that successfully sired offspring [31]. Thus, males may base their movement decisions on experience during past breeding seasons. Strikingly, however, males never flew west towards Russia with headwinds (figures 1 and 3). This corroborates a study at the Taimyr Peninsula, which reported that after 7 years without breeding records, nests were found in a year with easterly winds [47]. We speculate that this pattern is related to the autumn migration route. Pectoral sandpipers predominantly winter in South America [48] and all males that went to the Russian Arctic flew back east to Alaska and along the Canadian coast towards the Hudson Bay [27] (B.K. 2015–2019, unpublished data). Consequently, males that fly west into the Russian Arctic will later need more resources to cover a much longer migration distance, whereas males flying east into the Canadian Arctic were already moving in the direction of their later destination.

Assuming that time is an important resource [27,31], the observed 'loop' flights with long detours remain puzzling. We briefly discuss potential explanations. (i) Males 'overshoot' land during migration. Although a common phenomenon in bird migration [28], it seems unlikely because males already arrived (and started to compete) at a breeding site. (ii) Some, possibly naive, individuals use the wind to explore new breeding sites, but when failing to find land, turn around. Pectoral sandpipers are common vagrants [49] and hence males may have a tendency to explore. (iii) 'Loop' flights may be an outcome of group behaviour. Males typically fly in small groups (approx. 10–100 individuals, our personal observations, see also [28]). If only few individuals have a goal (i.e. have decided to return to a previous breeding area, see above), these individuals may act as 'leaders', pulling a group in a particular direction. However, if a group contains a mix of individuals that want to fly east and west, the group might initially head in an intermediate direction, i.e. northwards, but later split when the directional conflict becomes too large [50,51].

5. Conclusion

Our study shows that wind conditions influence the direction and the speed of flights of male pectoral sandpipers during the breeding season. Males seem to be highly flexible in where they go and the prevailing wind conditions can explain both within- and between-year variation in the distribution of males across their arctic breeding range. The importance of collective behaviour (group decisions) and the potential role of a small number of 'leaders' that decide to return to a previous breeding site remains to be studied. The observed patterns could also be driven by males following females, which themselves sample multiple breeding sites (B.K. 2018-2019, unpublished data) and were present at the time when males made the observed flights. Variability in wind patterns over the Arctic Ocean in combination with unpredictable breeding site quality could favour nomadic movements in a species with no mate fidelity and female-only parental care, or at least will not create the environmental prerequisites for wind-optimized flyways of fixed migration routes as found for example in the Northern Hemisphere [52].

The results of this study illustrate that for nomadic species—or more generally for species that are not site faithful—decisions about where to go could be influenced by the movement of the medium they travel through (wind in the case of birds), such that the costs of the movement (flight costs) can be reduced. If the distribution of the aimed for resources (e.g. safety from predators, food, mates) is unpredictable over a large spatial scale, individuals may benefit from using the energetically cheapest routes to travel to a potentially suitable site. When considering 'optimal migration theory', these movements initially mirror 'adaptive drift' [2], with the difference that nomadic animals only need to compensate such that they reach any potential site (e.g. pectoral sandpipers need to reach land when flying over the sea ice), whereas highly philopatric species will have to compensate drift completely to reach their particular goal. In this context, comparing the costs linked to movements to different parts of the species's (breeding) range can be highly valuable, because it can explain seasonal or between-year variation in local (breeding) density. This also implies that in species with low levels of philopatry, local fluctuations in numbers cannot be used to estimate population size. Our study also implies that estimating changes in population size based on local counts in a species with low levels of philopatry requires prior knowledge about the mechanisms underlying the distribution of the species across its range.

Data accessibility. The datasets generated and analysed for this study, including code used for statistical analysis and figure production, are available at https://osf.io/amd3r/.

Authors' contributions. B.K. and M.V. initiated the study; J.K. analysed the data with input from B.K. and M.V; all authors interpreted the results; J.K. and B.K. wrote the paper with input from M.V.

Competing interests. We declare we have no competing interest.

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Chapter 3 - Supplementary material

Wind conditions influence breeding season movements in a nomadic polygynous shorebird

Johannes Krietsch, Mihai Valcu and Bart Kempenaers

Supplementary video

https://www.youtube.com/watch?v=A-Q5J1wRBUA

Visualization of male pectoral sandpiper flights with the actual wind conditions and with wind support indicated (based on interpolated wind data, see Methods) in 2012 (0:00; n = 49 flights) and 2014 (0:51; n = 36 flights). Track colour indicates wind support (m/s), i.e. the length of the wind vector in the direction of the bird's flight (ground vector), calculated using wind data at 750 m altitude (see Methods). Positive values represent tailwinds (green) and negative values headwinds (red). The wind particles are created at random positions and their flow is based on the wind conditions at their position in 30-min intervals. The colour of the wind particles corresponds to the wind speed and is identical to the positive wind support scale. The grey-blue area indicates suitable breeding habitat within the known breeding range of the pectoral sandpiper. Note the running date and time, and the scale bar on the bottom right. Map projection: polar Lambert azimuthal equal area with longitude origin 156.65° W (Utqiaġvik) from Natural Earth (www.naturalearthdata.com).

Supplementary figures

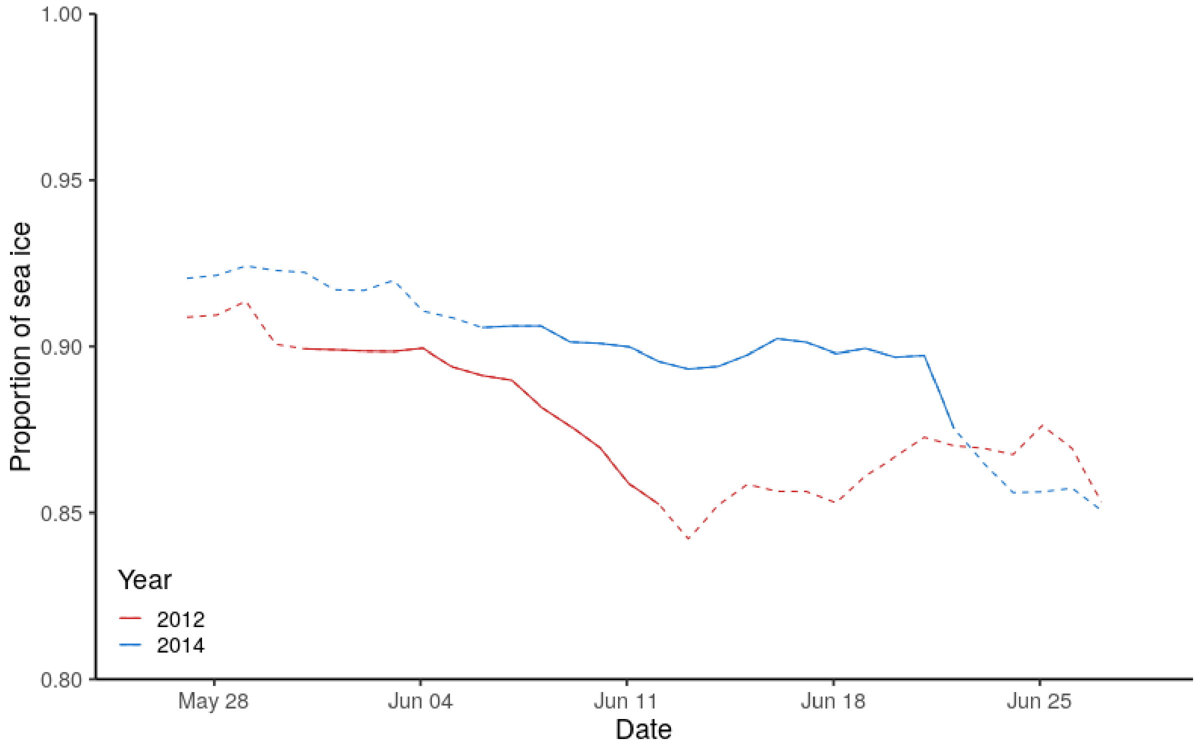

Figure S1. Proportion of sea-ice cover within the convex hull encompassing all tracks of male pectoral sandpipers (n = 85) over the ocean (see Methods) between 27 May and 27 June in 2012 (red) and 2014 (blue). Solid lines indicate the period between the first and last departure in each year. Sea ice data were obtained from the National Snow & Ice Data Centre (Fetterer et al., 2010), which provides daily information on sea ice cover with a 4 km resolution. Most of the ice-free ocean was located west or south-west of Banks Island.

Reference:

Fetterer F, Savoie M, Helfrich S, Clemente-Colón P. 2010. updated daily. Multisensor Analyzed Sea Ice Extent - Northern Hemisphere (MASIE-NH), Version 1. Boulder, Colorado USA. NSIDC: National Snow and Ice Data Center. doi: 10.7265/N5GT5K3K.

Figure S2. Prevailing wind direction (in % of the time) and wind speed at ~750 m (most likely flight altitude) within the area of all male tracks (n = 85) over the ocean (a, b) and over land (c, d) on days with actual departures in 2012 (a, c) and 2014 (b, d). For every day the mean wind speed and direction within the area was calculated (see Methods). Over the ocean, wind direction did not vary significantly in mean between years (Rao's Tests for Equality of Polar Vectors = 0.02, *p* = 0.87), but in dispersion (Rao's Test for Equality of Dispersions = 106.94, *p* < 0.001). In general, wind speed was higher over the ocean than over land (difference: 1.61 m/s ± 0.37 s.e., *p* < 0.001) and higher in 2012 compared to 2014 (difference: 1.15 m/s ± 0.37 s.e., *p* = 0.003; linear mixed-effect models with departure night (scaled by year) as random effect). In the legend, a square bracket indicates that the value is included, a round bracket indicates any value higher than the indicated value.

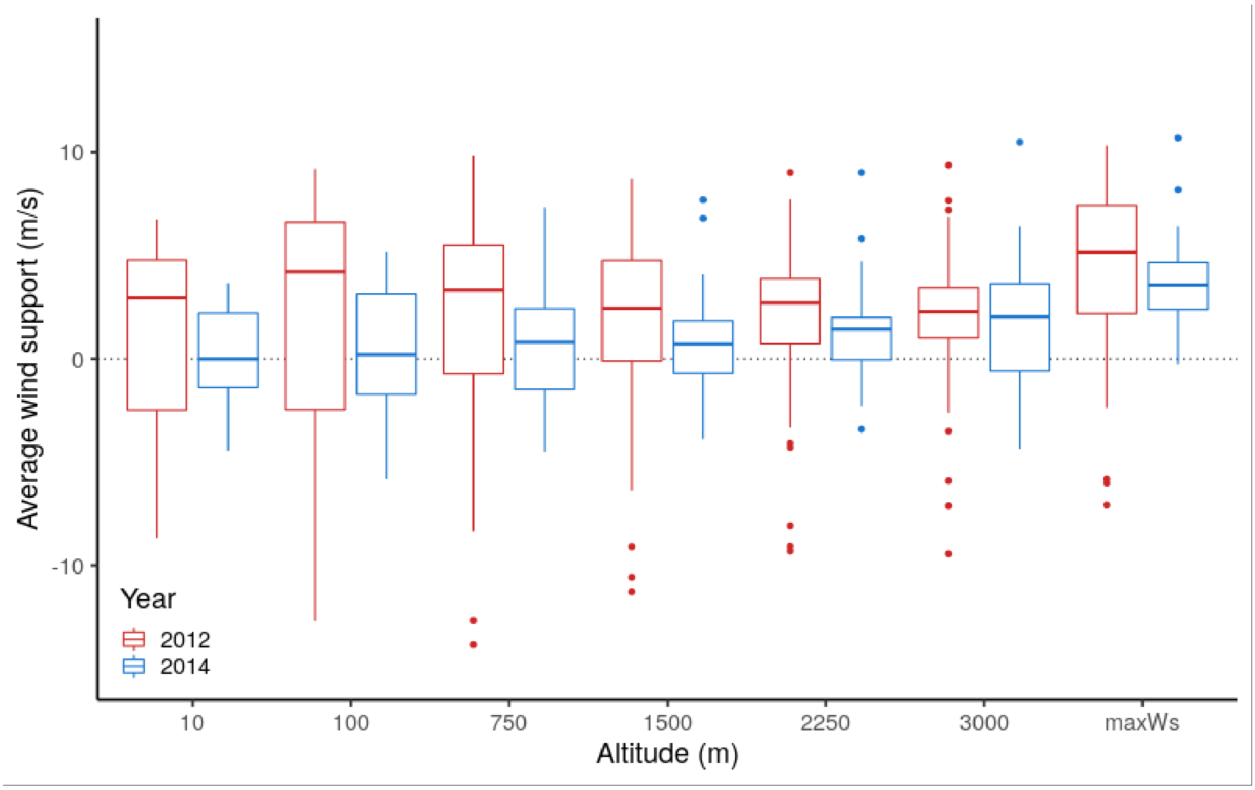

Figure S3. Mean wind support for male pectoral sandpipers that departed around Utqiaġvik, Alaska in 2012 (n = 49) and 2014 (n = 36) for different flight altitudes. The altitude with maximal wind support (maxWs) was calculated based on the highest mean wind support in each 1 h flight bout (from among the six fixed altitudes). Mean wind support was significantly different between 10 m and 2250 m, between 10 m and 3000 m, and between 100 m and 3000 m (based on a linear mixed-effect model with mean wind support as the dependent variable, altitude and year as fixed factors, and individual ID as random intercept; Tukey test to compare different altitudes). The mean wind support of the maxWs altitude was significantly higher than the mean wind support of any fixed altitude (based on the same model).

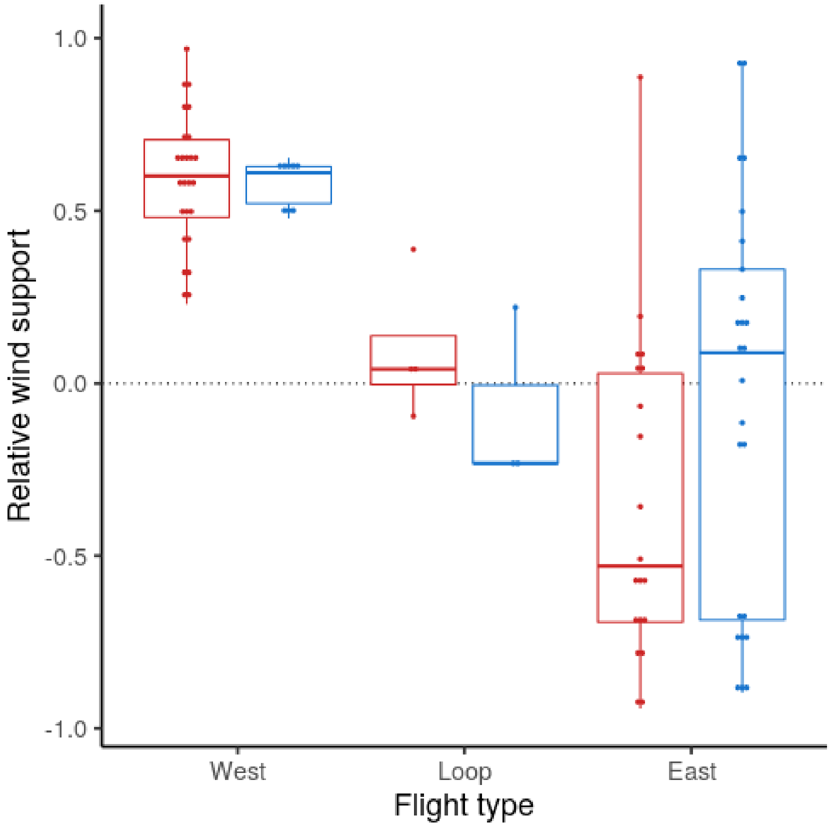

Figure S4. Average relative wind support for the entire flight in relation to flight type and year. Relative wind support is defined as wind support (calculated based on wind data at ~750 m altitude, see Methods), divided by wind speed at each location. Loop flights are defined as tracks with a high straightness (median = 7.8). Westward and eastward flights are defined as tracks directed toward their respective destination with low straightness (see Methods). Shown are box-plots with median (centre line), 25-75th percentile (limits), minimum and maximum values without outliers (whiskers), and outliers (dots). The number above the box indicates the number of tracks in each category.

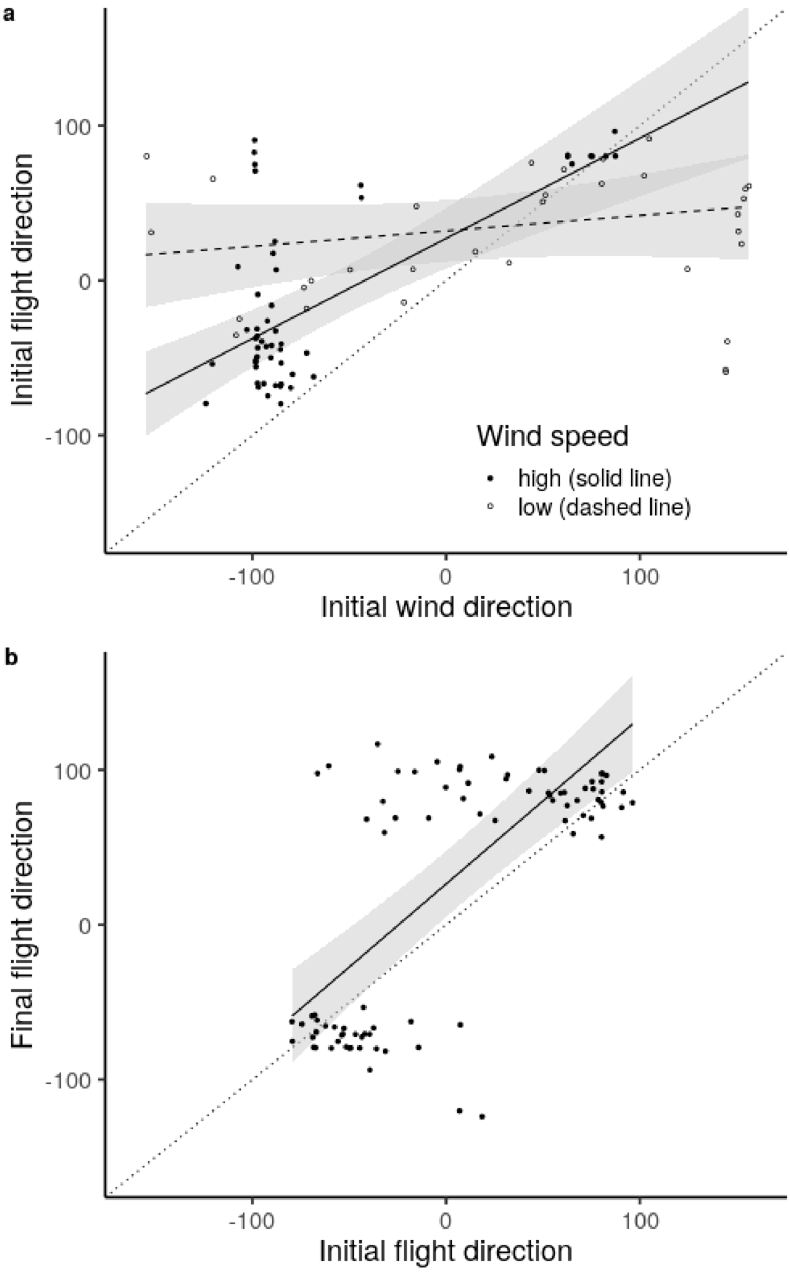

Figure S5. (a) Initial flight direction of male pectoral sandpipers (0 = north, mean of the first 50 km) in relation to the initial wind direction (mean wind direction during the first 50 km of the flight at ~750 m altitude), overall (solid line) and for different wind speeds (low: <5 m/s, high: >5 m/s). Data from 2012 (n = 49) and 2014 (n = 36). (b) Final flight direction (the direction from the departure to the arrival location) in relation to the initial flight direction. Shown are model estimates (lines) and 95% confidence intervals (grey areas). See electronic supplementary materials tables S4-S5 for model descriptions.

Supplementary tables

Table S1. Most likely flight altitude based on a linear mixed-effect model of ground speed (m/s) explained by wind support and crosswind for each altitude with track ID as random effect. The altitude with maximal wind support (maxWs) was calculated based on maximum wind support (see Methods). To control for temporal autocorrelation we used a moving-average correlation structure. Models were fitted with maximum likelihood and ranked based on the AIC criterion. For the summary statistics of the best fitting model see Table S2.

Altitude	AICc	delta AIC
750 m	14997.03	0
1500 m	15002.04	5.0
2250 m	15023.86	26.8
maxWs	15026.14	29.1
100 m	15039.41	42.4
3000 m	15046.22	49.2
10 m	15047.66	50.6

Table S2. Influence of wind support and crosswind on the ground and air speed of male pectoral sandpipers (n = 85 tracks). Shown are results from a linear mixed-effect model with ground speed (m/s) or air speed (m/s) as the dependent variable, with wind support and crosswind (at ~750 m altitude) as explanatory variables and with track ID as random intercept. To control for temporal autocorrelation we used a moving-average correlation structure.

Dependent variable	**term**	**Estimate**	**SE.**	**Statistic**	***p***
Ground speed	Intercept	14.63	0.28	53.10	
	Wind support	0.31	0.03	9.17	<0.001
	Crosswind	-0.06	0.03	-1.72	0.27
	Wind support x crosswind	-0.02	0.00	-5.61	<0.001
	Track ID (variance)	0.00			
	Residual variance	5.18			
Air speed	Intercept	14.73	0.24	61.06	
	Wind support	-0.58	0.02	-24.32	<0.001
	Crosswind	0.26	0.03	8.64	<0.001
	Track ID (variance)	1.13			
	Residual variance	4.07			

Table S3. The relationship between track length and mean wind support (WS, in m/s) during the first half of the flight (n = 85 tracks). We fitted a linear model using wind data at ~750 m altitude and included category as fixed factors to correct for the effect of different potential flight lengths based on direction.

Term	**Estimate**	**SE**	**Statistic**	***p***
Intercept	1150.71	57.95	19.86	
Category (loop)	299.47	163.50	1.83	0.22
Category (west)	315.35	115.53	2.73	0.028
Mean WS first half	21.73	9.85	2.21	0.030

Table S4. Influence of wind direction (degrees) in combination with wind speed (m/s) during the first 50 km on the initial flight direction (mean over the first 50 km) for male pectoral sandpipers that left Utqiaġvik, Alaska (n = 85 tracks). Shown are results from a linear mixed-effect model with initial direction (degrees) as the dependent variable, with an interaction of wind direction and wind speed (at ~750 m altitude) as explanatory variables and with date (night in a given year) as random intercept.

Model	**Term**	**Estimate**	**SE**	**Statistic**	***p***
Continuous wind speed	Intercept	18.35	10.06	1.82	
	Wind direction	-0.01	0.09	-0.13	1.00
	Wind speed	2.31	1.63	1.41	0.39
	Wind direction * wind speed	0.07	0.02	4.16	<0.001
	Day (variance)	11.26			
	Residual variance	40.86			
Categorised wind speed	Intercept	27.18	9.86	2.76	
	Wind direction	0.65	0.11	5.78	<0.001
	Wind speed low	4.86	12.92	0.38	0.98
	Wind direction * wind speed low	-0.55	0.14	-4.05	<0.001
	Day (variance)	21.15			
	Residual variance	38.07			

Table S5. Relationship between the final flight direction (the direction from the departure to the arrival location) and the initial flight direction (average of the first 50 km). Shown are results from a linear mixed-effect model with final flight direction (degrees) as the dependent variable, initial flight direction as explanatory variable and with date (night in a given year) as random intercept.

Term	**Estimate**	**SE**	**Statistic**	***p***
Intercept	26.30	10.70	2.46	
Initial direction	1.07	0.13	8.51	<0.001
Day (variance)	42.17			
Residual variance	48.31			

Table S6. Results of a binominal model of the number of departures of male pectoral sandpipers going west (n = 36) and east (n = 45) against initial delta wind support (mean wind support for going west minus mean wind support for going east for the first 50 km). Date (the night of departure in each year) is included as random effect. The wind support (at ~750 m altitude) for every night was estimated by using all tracks of birds that went either west or east, shifting their departure to the average departure hour (22:30 h) for each night (see Methods).

Term	**Estimate**	**SE**	**Statistic**	***p***
Intercept	-1.37	0.61	-2.23	
Initial delta wind support	0.12	0.04	2.80	0.009
Day (variance)	0.67			

Table S7. Results of a linear mixed-effect model of relative wind speed explained by relative track length and wind speed (m/s) using track ID as random effect (n = 85 tracks). Relative wind support is defined as wind support (calculated based on wind data at ~750 m altitude, see Methods), divided by wind speed at each location. A relative wind support of one corresponds to a heading that is identical to the direction of the wind, independent of the wind speed; a relative wind support of minus one corresponds to a bird flying entirely against the wind. To allow comparison between tracks of different length (min = 584 km; max = 2609 km), we show relative track length (dividing by total track length).

Term	**Estimate**	**SE**	**Statistic**	***p***
Intercept	0.16	0.08	2.09	
Relative track length	-0.38	0.08	-4.44	<0.001
Wind speed	0.03	0.00	5.63	<0.001
Track ID (variance)	0.35			
Residual variance	0.59			

Table S8. Results of a linear model with mean wind support by track as response variable and category (east, west and loop) and year as fixed factors. Wind support between each flight category was compared with a post-hoc Tukey test.

Term	**Estimate**	**SE**	**Statistic**	***p***
Category (west - loop)	4.23	1.40	3.02	0.009
Category (west - east)	6.58	0.81	8.17	<0.001
Category (loop - east)	2.35	1.37	1.72	0.20
Year (2014)	0.87	0.77	1.12	0.58

Table S9. Differences in wind support depending on shifts in departure date and time. Results from linear mixed-effect models of delta wind support (real-time track minus time-shifted track) of male pectoral sandpipers separated in birds going west (n = 33), east (n = 45) or loops (n = 7). Each track was shifted in 24 h intervals for ±5 days and in 1 h intervals for ±12 h. *p*-values indicate whether the difference in wind support between the observed and shifted time was significantly different from zero, i.e. whether birds would on average have gained or lost wind support by departing at a different day or time. We used track ID and day (night of departure in each year) as random effects.

Model	**Term**	**Estimate**	**SE**	**Statistic**	***p***
Days	Shift to later	-0.01	0.01	-1.19	0.65
	Category (east)	0.59	1.31	0.45	0.99
	Category (loop)	0.47	2.47	0.19	1.00
	Category (west)	3.55	1.54	2.31	0.09
	Shift to earlier	1.05	0.91	1.15	0.69
	Night (variance)	5.04			
	Track ID (variance)	4.08			
	Residual variance	5.67			
Hours	Shift to later	0.00	0.01	-0.47	0.99
	Category (east)	-0.02	0.18	-0.09	1.00
	Category (loop)	-0.04	0.31	-0.12	1.00
	Category (west)	0.16	0.20	0.78	0.90
	Shift to earlier	0.06	0.12	0.54	0.98
	Night (variance)	0.59			
	Track ID (variance)	0.62			
	Residual variance	1.22			

Table S10. Results from linear mixed-effect models of (a) the difference in wind support (delta WS, in m/s, at ~750 m altitude) between the actual track and the shortest route and (b) the difference in flight time (delta time) for the actual versus the shortest route in relation to track category. Loop flights are defined as tracks with high straightness (median = 7.8). Westward and eastward flights are defined as tracks directed toward their respective destination with low straightness (see Methods). In both models, date (the night of departure in each year) was included as random effect.

Dependent variable	**Term**	**Estimate**	**SE**	**Statistic**	***p***
Delta WS	Category (west)	-1.53	0.87	-1.75	0.20
	Category (loop)	3.51	1.31	2.69	0.020
	Category (east)	2.05	0.76	2.71	0.019
	Day (variance)	3.01			
	Residual variance	2.58			
Delta time	Category (west)	0.53	1.22	0.43	0.96
	Category (loop)	23.28	2.43	9.58	<0.001
	Category (east)	2.57	1.02	2.53	0.034
	Day (variance)	1.73			
	Residual variance	6.11			

General discussion

In this dissertation we did explorative research that was driven by our interest in previous descriptions of the species and long standing hypotheses, as well as the eagerness to discover new things. We did this by a combination of classical behavioural observations, genetic methods for paternity assignment, and the latest tracking technologies. This combination allowed us to describe the social and genetic mating system of the sex-role reversed red phalarope in newfound comprehensive detail and allowed us to study the influence of the prevailing wind conditions on the breeding site sampling behaviour of the polygynous pectoral sandpiper. In this general discussion, I will start by providing a general description of the mating system of both species, with a main focus on red phalaropes. I will then follow with a comparison between both species, to review their mating behaviour and reproductive success optimization strategies in the general context of sexual selection. Finally, I will discuss about how environmental conditions influence polygamy, mate searching and mate choice in both species.

The mating system of red phalaropes and pectoral sandpipers

Red phalaropes and pectoral sandpipers are sympatric species that fall at the opposite extremes in terms of intrasexual selection, which creates both bold similarities and sharp differences in their mating systems. In the following sections I want to describe the characteristics of their mating systems and how this study contributed to their understanding.

Mating system of red phalaropes

We found lower rates of social polyandry in red phalaropes than previous studies suggested. On average 7% of females (11/162 over three seasons) mated with multiple males. In 2017, only one female (3% of 34) laid clutches for two males. Intriguingly, this female laid both her first and second clutch even before any female in the study site laid its first and only clutch. In 2018, two females (5% of 37) and in 2019 eight females (9% of 91) were socially polyandrous. Two of these females even laid three clutches, the latest one of which was in both cases the replacement of a failed second clutch with the same mate. The rates of social polyandry that we documented are lower than the one of previously findings: 44% (4/9; Schamel and Tracy, 1977) and 50% (3/6; Whitfield, 1995). Given a much larger study site and effort (i.e. number of observers and additional use of tracking devices), we actually expected to find higher rates of social polyandry.

So, what could explain this disparity? While there is no doubt that the detailed observations by Schamel and Tracy (1977) and Whitfield (1995) reported correct numbers, the small sample sizes and particularities of their study sites, might have resulted in estimates of the rate of polyandry that are potentially not representative for the larger population or species. This is especially true for the study of Whitfield (1995), which was done on a small population in Iceland over two years. This population consisted only of three females and five males in 1986 and three females and eight males in 1987. The study of Schamel and Tracy (1977) was instead based on the “same” red phalarope population that has been investigated in our work, namely in the area around Utqiaġvik but more than 40 years earlier (1974 and 1975 vs. 2017-2019). While the exact location of the study site from Schamel and Tracy (1977) is not clear, they describe that it was close to a regularly-travelled road south of NARL (potentially Cakeeater road, see Figure 3 in general introduction). The habitat next to this road is one of the first snow-free areas around Utqiaġvik because the dust from the frequently used road speeds up the snow melt. Our study site also comprised a small section of road close to the BARC. Here the snow melted significantly earlier and created a small wet area with excellent foraging habitat for shorebirds early in the season. While this was happening, our study site offered very scarce opportunities to breed, since this wet area provides little breeding habitat and the rest of the tundra was still mostly snow covered. Given that we found that particularly early breeding females to be the ones with the highest chances to become polyandrous (Chapter 1), Schamel and Tracy’s study site likely provided better chances for females to become polyandrous. However, their estimate for the rate of polyandry is also only based on observations on nine females. Small-scale local differences in snowmelt patterns and potential uncertainties due to a small sample size might therefore explain the differences in the rates of social polyandry between our and Schamel and Tracy’s study.

The rates of polyandry we observed are similar to those reported for the closely-related red-necked phalarope *Phalaropus lobatus*, which are based on sample sizes similar to ours (8% over five years, n = 59; Reynolds, 1987; and 9% averaged over eight years, range: 5-16%, exact sample size unclear, but likley >100 females Schamel et al., 2004b). Nevertheless, our estimate of the proportion of polyandrous females includes two extremely late and short breeding seasons (2017 and 2018). The exceptionally late snow melt in these years limited habitat availability and the emergence of insects (McGuire et al., 2020). As a consequence, relatively few individuals attempted to breed and only three females where knowingly polyandrous in these two years (Chapter1). Nevertheless, we might underestimate the rates of polyandry, since males and females are non-territorial and move over a much larger area than our study site. We found one female that laid clutches with 2.2 km distant from each other and we recorded movements far away from our study site (Chapter 1), suggesting potential for higher rates of polyandry when considering larger areas (see Outlook below).

From the genetic perspective, we found 11% (37/334) of the clutches contained at least one extra-pair young. Therefore, the rates of genetic polyandry were also lower than those reported in the only other parentage study on red phalaropes, which found extra-pair paternity in 33% (6/18) of the clutches (Dale et al., 1999). These are relatively low rates of extra-pair paternity when compared to most monogamous bird species, particularly passerines (Brouwer and Griffith, 2019). Nevertheless, 11% is still comparably higher than in most socially monogamous shorebirds with biparental care (Chapter 1, Table S1). Yet, lower when compared to simultaneous polyandrous jacanas (Emlen et al., 1998; Haig et al., 2003), where females copulate with multiple "harem" males while producing eggs for one of them. In contrast, red phalaropes are only paired to one mate at the time and in this way can be seen as serially monogamous within a breeding season.

So, what describes the mating system of red phalaropes best then? Throughout the general introduction I introduced multiple terms and all of them fit: Classical polyandry, sequential polyandry, female access polyandry, facultative polyandry, social polyandry and genetic polyandry. Given the potentially low rates of social polyandry in some populations and in some years, this mating system can at times also be described as social or serial monogamy (between breeding seasons), as the majority of females just lay for one mate. It must be noted that we even identified one pair that bred together in two consecutive years, but this is certainly an exceptional observation. To add more confusion to all these terms, they are not always used consistently with the same meaning. In my opinion, the best approach when describing the mating system of a species is to give a short summary of how it actually works, to allow the reader to get a clear picture of it. For example, for red phalaropes this would be as follows: socially polyandrous, non-territorial species with male-only care; males and females form short-term pair bonds, that typically terminate during or soon after laying, after which females try to find a second mate; if additional mates are available, females can lay a sequential clutch with a new mate, but most females typically only lay one clutch; rarely, females were observed laying a third clutch in order to replace the second one after it had been lost, and this third clutch was again cared for by the second mate.

Mating system of pectoral sandpipers

While a description of the mating system of pectoral sandpipers is not the focus of this study, I would like to briefly discuss it to allow a meaningful comparison of the two species. Pectoral sandpipers are polygynous with female only care. Males set up display territories that typically contain breeding and foraging habitat, while females can move between male territories freely and may choose to breed in one (Farmer et al., 2020). Males and females form no pair bond, but males defend females within their territory. Males can sire offspring with multiple females, which can lead to up to 22 young sired in total locally, but most males might only sire offspring in one clutch and some no offspring at all. However, the reproductive success of males is nearly impossible to quantify because of their large-scale movements within one breeding season. Males visit often multiple sites (up to 24 observed) in which they likely set up territories too (Kempenaers and Valcu, 2017). Most females are genetically monogamous, but 16% of 170 clutches were sired by two males (Kempenaers and Valcu, 2017).

Sexual selection in red phalaropes and pectoral sandpipers

After describing the mating systems through the numbers of social and genetic mates, I want to now focus on the selective drivers and strategies to increase reproductive success which result in the above described pattern and are ultimately essential to understand the mating system.

Mate monopolization strategies

One key aspect in understanding polygamous mating systems is the process that allows males or females to access multiple mates (Emlen and Oring, 1977). Let's take a closer look at how female red phalaropes and male pectoral sandpipers try to do that. In red phalaropes female mate monopolization is achieved through mate guarding of one male at a time (Chapter 2). Females stay almost continuously in close proximity to the male, from the moment of pair formation until at least the first egg is laid. Physical fights between females can likely lead to mate changes before the pair bond is established, because also males engage in fights with females that come too close to the pair. In most cases the association between pairs dissolves during the egg-laying period. In some cases, females already start associating with a subsequent male, while still laying eggs for the first partner (Chapter 1 and 2). This allows females, if they can find another male, to lay two clutches in immediate succession.

Based on our study, females that lay their first clutch early in the season have the best chances to find a second mate (Chapter 1). This suggests that females compete to lay early, to increase their chances of becoming polyandrous, which can be advantageous when the local adult sex-ratio is male biased. In such situations females that first re-join the mating pool have the best potential to monopolize a second male (Andersson, 2004). That early breeding increases the potential to become polyandrous was also shown in Kentish plovers (McDonald et al., 2022) and snowy plovers (Eberhart-Hertel et al., 2023). In both species, females are more likely to desert the chicks with increasing prospect of finding a subsequent mate (Kupán et al., 2021; McDonald et al., 2022). However, note that both species have biparental incubation and females or males facultatively desert the chicks after hatching.

Why do red phalarope females not defend multiple males at the same time? This is for example the case in socially polyandrous jacanas, which can defend territories with sub-territories of multiple males (Butchart, 1999; Emlen et al., 1998; Fresneau et al., 2021; Haig et al., 2003). I think this difference to jacanas is linked to the limited resource monopolization potential of red phalaropes. Food for red phalaropes is spatially clumped (i.e. small ponds), and it is often ephemeral and attracts high densities of individuals, which makes it impossible for single females to defend a territory in general, and in particular with multiple males inside. Furthermore, males also invest in mate guarding, and prevent the female from interacting with other males (Chapter 1), limiting the options for females to engage with multiple males simultaneously. This close association (i.e. pair bond), which is characterized by mutual mate guarding and which is focused on direct monopolization of one mate without monopolization of resources, is probably linked to the non-territorially of this species.

In contrast, male pectoral sandpipers defend territories, that typically contain suitable breeding and foraging habitat (Lesku et al., 2012; Pitelka, 1959). This resource defence does not necessarily allow female monopolization, since females are free to move between territories (Kistchinskii, 1974; Pitelka, 1959). However, moving between male territories is potentially costly for females, since males will heavily court females that enter their territory and attempt to copulate with them. Therefore, a female could benefit from the protection provided by the territory holder against harassment from other males. This hypothesis still needs formal testing, but low rates of mixed paternity could indicate limited female movements. Mixed paternity, could then particularly arise by male take-over of a territory during the fertile period of the female, or still by females visiting multiple male territories. Future studies on female movements between male territories and the identity of sires could shed light on the underlying causes.

The best strategy to increase mate access for male pectoral sandpiper might then be to defend a territory with good foraging and breeding habitat at a time when many fertile females are around. This creates intense male-male competition, with fights that can sometimes even result in open wounds. However, while fights do occur regularly, most disputes are solved non-aggressively with displays (i.e. “parallel walks” or “parallel flights”). This intense male-male competition even leads to adaptive sleep loss, as males that sleep the least sire more offspring (“you snooze, you lose"; Lesku et al., 2012). From my observations, part of the differences in activity is probably linked to different durations of males holding territories, as males that “give up” on a territory reduce activity and forage in exclusive foraging sites. These males then likely have limited chance to sire offspring. Another non-exclusive effect could be simply the presence of fertile females, which itself increases competition and activity. The latter is indirectly supported by the observation that males stay only for a short time, if only few fertile females are present (Kempenaers and Valcu, 2017). In this way, pectoral sandpiper males adjust local effort to reproductive prospects and try to maximize access to females by finding sites with the best potential to successfully compete (Kempenaers and Valcu, 2017). This strategy works best in asynchronous breeding seasons in which males could make use of several sites with local optima, i.e. with high numbers of fertile females and potentially a female-skewed operational sex ratio. However, the conditions of distant breeding sites are likely unpredictable and chance might play a major role when moving nomadically through the breeding range.

To summarize, red phalarope females try to mate with multiple males sequentially, mainly by indirect competition to lay earlier, while pectoral sandpiper males compete directly for territories and rely on the territories and on courtship displays to attract females. Individuals of both species can increase the number of mates in long and asynchronous breeding season, and can potentially increase their chances of becoming polygamous at sites with skewed operational sex ratio. Red phalarope females might be limited to two or maximally three social mates (as some individuals have the potential to lay three clutches), while pectoral sandpiper males might sire offspring with six or more females. The mating skew is therefore expected to be higher in pectoral sandpipers and sexual selection is expected to be stronger (Kempenaers and Valcu, 2017) than in red phalaropes. An indication for the stronger sexual selection is the magnitude of sexual size dimorphism in both species: Red phalarope females are on average 23% heavier than males, while pectoral sandpiper males are on average 45% heavier than females (own data, Dale et al., 2007).

Pair bond vs. short association

Given this strong intrasexual selection in red phalaropes and pectoral sandpipers the association of males and females in both species is, in a certain sense, reduced to a minimum. Still red phalaropes form a short, but strong pair bond, while pectoral sandpipers do not socially associate with their mate. In this section I want to discuss what a pair bond is, why I think red phalaropes have a pair bond and pectoral sandpipers do not and why this is the case.

What is a pair bond? As with many definitions in biology, the term is often defined in different ways often depending on the taxonomic group. Ultimately, it is a psychological construct characterised by behavioural and physiological measurements. Recently this issue was reviewed by Bales et al. (2021), who presented how the term is commonly used and attempted to unify concepts by developing a definition applicable to all species. Apart from one point, regarding to the length of the pair bond, I agree with the authors’ proposed definition and I will now go through the points for red phalaropes. The proposed definition contains points the association between individuals must fulfil, may fulfil and cannot fulfil.

Points that must be fulfilled: Pair bonds (1) must be between two sexually mature individuals and (2) specific to the individuals that are involved (i.e. individuals cannot readily be replaced). This is clearly true in red phalaropes. Pair bonds (3) must contain an affective component. I would classify the “pushing displays” of red phalaropes as affiliative behaviour, which is characterised by males and females pressing their breasts against each other, often crossing their bills while both perform soft twittering calls (Bengtson, 1968). This behaviour is part of the pair formation and often performed in the presence of other conspecifics. Moreover, pair bonds (4) must have a measurable degree of strength or quality. Pair bond strength or quality in red phalaropes can be assessed by measuring the time spent together per day (Chapter 2). According to Bales et al. (2021), pair bonds also (5) must persist beyond one reproductive cycle. This is the point I disagree with, because it would not allow animals to be defined as a social pair if they reproduce only once together, which could also be the case if one of the individual dies after the first reproductive cycle. I would change this aspect of the definition to: Pair bonds must have the potential to persist for multiple reproductive cycles. While red phalaropes typically do not reproduce with the same mate again, due to their low site fidelity, pairs can re-nest if the first clutch fails (e.g. due to predation). In one exceptional case, we even observed a pair breeding together in two consecutive breeding seasons. I think it makes sense to classify the social association of red phalaropes as pair bond because of their close social association, even though they usually do not associate for multiple reproductive cycles.

Points that may be fulfilled: Pair members (1) may engage in sexual behaviours, and they (2) may be of the same, different or mixed sexual orientation. We only observed pair bonds between males and females in red phalaropes. These are characterised by high copulation rates. Pair members (3) may participate in biparental care. This is not the case in red phalaropes which have male-only care, but biparental care is not a necessary criterion of the definition of a pair bond. (4) Pair members may display coordinated behaviours. This can be observed in red phalaropes in the form of “parallel swims” and “pushing displays” (Bengtson, 1968).

Points that cannot be fulfilled: Pair members (1) cannot be asocial and (2) have to be able to identify each other among others. Red phalaropes are clearly able to identify each other and can repeatedly reconnect after being separated for some time (Chapter 2). Pair members (3) cannot drive away or kill the mate directly after mating. Such behaviours do not happen in red phalaropes. However, females might just leave their partner directly after laying the last egg of a clutch. Pair members (4) cannot be indifferent to separation from the mate. We could observe clearly that red phalaropes were distressed when separated from their mate when we only caught one of the pair members. In such instances, the other pair member would immediately start loud calls and fly in circles to search the mate.

Taken everything together, I think red phalaropes clearly have a strong pair bond, even though it is really short (only around a week in some cases), and in fact this might even be one of the shortest pair bonds among all birds. Pectoral sandpipers do not fulfil the definition of forming a pair bond, as they are not social, associations are not specific to one individual, have no affective component, and mates appear indifferent to separation. Associations between males and females might in some cases just be one copulation, but females might also be guarded if they remain in the male territory.

I think there is a physiological reason for the different types of associations between red phalaropes and pectoral sandpipers, which is that females lay eggs. Females are certain of their maternity, but males cannot be so certain of their paternity, as females might engage in extra-pair copulations or might have copulated with other males before bonding. This means that in competition for mates, male pectoral sandpipers could reduce their parental investment uniquely to the act of copulation, leaving females to do all parental care without getting more than sperm. Red phalarope females, in contrast, cannot just lay eggs for any male and “expect” this male to immediately take care of the clutch. Males have to be “reassured” to some degree of their paternity in order to provide all parental care. Therefore, males and females have to spend some time together before clutch initiation, which is characterised by a pair bond with frequent within-pair copulations and intense mate guarding (Chapter 1 and 2).

In this way, a pair bond is simply a shared “commitment” to one or multiple reproductive events. This mutual “commitment” is initially expressed through affiliative behaviours with indicate the willingness of male and female to invest in reproducing together. Both sexes invest into mate guarding, deterring same-sex individuals and in frequent copulations (which are mainly costly for males). Interestingly, while in a sex-role reversed species the female is typically expected to initiate pair formation, male red phalarope are sometimes initiating pair formation and actually compete for females. This can happen with a male-biased operational sex ratio, which makes females the limiting sex (Tracy and Schamel, 1988). Even while males and females are paired, differences in mate availability can result in different investment into mate guarding. Mate guarding can be more driven by males early in the season, while shifting to being more female-driven later in the season, when the operational sex-ratio will be female biased (Chapter 2).

To summarize, intrasexual selection in red phalarope females and pectoral sandpiper males lead to the shortest mate association possible in both species, to allow individuals to re-join the mating pool again as fast as possible. Sexual conflict is higher in red phalaropes, as male reproductive success will decrease with loss of paternity. This creates selection pressure for males to only accept a clutch after a certain time of association (i.e. a pair bond characterised by frequent copulations and mate guarding). Males' scraping behaviour (nest preparation), might then indicate their willingness to accept a clutch.

Mate choice and cryptic female choice with stored sperm?

Now that we had a closer look at intrasexual selection and the association between males and females in red phalaropes and pectoral sandpipers, in this section I want to discuss mate choice (intersexual selection) in both species. Sex roles determine which sex is expected to be choosier, which are red phalarope males and pectoral sandpiper females. However, the more competitive sex could be choosy in some way as well. In particular, in red phalarope females, mate choice could happen post-copulatory based on sperm that is stored.

Most red phalaropes already arrived paired at our study site, which limited our possibility to do empirical analysis on this topic. Nonetheless, behavioural observations at the beginning of the season gave some insights. At the first snow-free sites around Utqiaġvik males and females arrived simultaneously. At this point pair bonds were not necessarily established and there were whole groups of birds that foraged in ponds. Most pair formation behaviours (i.e. "pushing displays" and "parallel swims", but also copulations) were observed in this period. At this early point of pair formation, males might also reject to copulate with females. I vividly remember seeing a female that presented her tail and waved it at a male, who simply ignored her solicitation behaviour. In contrast, the sexual drive of male pectoral sandpipers is so extreme, that they can regularly be observed mounting even other species, like dunlins *Calidris alpina*, semipalmated sandpipers *Calidris pusilla*, curlew sandpipers *Calidris ferruginea*, and one male was even observed mounting a dead red phalarope male (Farmer et al., 2020 and own observations). In the case of curlew sandpipers, this regularly results in hybrids (hybrids were initially thought to be a new species, which was called "Cox's sandpiper"). Male pectoral sandpipers are therefore apparently not choosy at all.

One could think that male red phalaropes should also use every opportunity to copulate with females, since even if they do not end up pairing up, he might sire extra-pair offspring. So, why might they reject solicitations to copulate? Some males might not be physiologically ready to copulate or the potential chance of gains (i.e. siring extra-pair offspring) might be too low at this early time in the season. A copulation several days before egg-laying will unlikely result in any fertilization, as females will have many copulations with their social mate before egg-laying. If sperm is a limiting resource for males, they might also invest more in copulations with their own social male to increase the chances of paternity in the clutch they care for. Nevertheless, males might readily copulate with other females that are close to egg-laying when chances to sire extra-pair offspring are higher. Moreover, they could actively seek extra-pair copulations once they have secured paternity in their own clutch. At this point, in fact, they do not need to mate guard their social female anymore and, if they are not incubating yet, their sperm production might still be high. Around the clutch initiation peak males have the highest chances to sire extra-pair offspring, when many fertile females lay almost synchronically. Additionally, when females are close to completion of their first clutch, they will begin to search for a second mate and they will solicit copulations in an attempt to start a pair formation process. Males that are close to the start of incubation of their own clutch might use this situation (of females seeking a second pair bond) with the prospect of siring extra-pair offspring (Chapter 1). Therefore, red phalarope males are expected to be choosy, depending on the prospects to sire offspring. This concept of context-dependent male mate-choice is also predicted by a theoretical study on mate choice in polyandrous species (Puurtinen and Fromhage, 2017).

Male mate choice for a social mate could also be influenced by preferences for physical traits (e.g. regarding size or coloration). This could play a role when males have a direct choice between two or more females, but this remains to be empirically tested. Once a male invested in a social female (i.e. copulations and mate guarding) it is unlikely he would change mate, because even a better mate would unlikely outweigh the investment (and thereby the gains in certainty of paternity) that he already made. The highest potential for mate choice is therefore expected in a situation when males try to find a female and have the choice between multiple females at the same time (e.g. due to a female-biased operational sex ratio). When the operational sex ratio is however male biased, males should even compete for females, and then females have theoretically a higher potential to choose (Tracy and Schamel, 1988). With a female biased operational sex ratio, I do not expect much female choice, but rather expect females to try to bond with whatever male they find. Mate choice can therefore be more pronounced in either male or female in red phalaropes, depending on the sex ratio.

Indirect female choice for “good gene” benefits was also suggested via the “sperm-storage hypothesis” for red phalaropes and for sequentially polyandrous birds in general (Dale et al., 1999; Oring et al., 1992; Schamel et al., 2004b). With females potentially excluded from the opportunities of extra-pair copulations, these studies suggested that polyandrous females might store sperm for extended periods in sperm storage tubules (Birkhead and Møller, 1993; Frey and Goymann, 2009; Holt and Fazeli, 2016) to increase the probability of fertilizations in subsequent clutches. These studies suggested that first mates should generally be of higher quality, given that older and more competitive males will arrive earlier at the breeding grounds and females should therefore benefit from additional offspring form the first males in their second clutch (Oring et al., 1992). While we found higher rates of extra-pair paternity in second clutches of polyandrous females in our study (27% in 11 clutches compared to 0% in 15 known first clutches and 10% in 138 potential first clutches; Chapter 1), I think that direct benefits of mate acquisition behaviour of females is sufficient to explain these differences in the rates of extra-pair paternity, without requiring potential indirect benefits of cryptic female choice. Females can gain more from acquiring a (second) mate in general, than they could from "good gene" benefits (Kirkpatrick and Barton, 1997) and therefore they actively solicit males in an attempt to initiate the pair formation process. Some males might use this situation to copulate with females without the intention to care for a clutch. Hence, overall, extra-pair paternity in red phalaropes is readily explained by the sequential social pairing, and there is no need to invoke any strategic sperm retention for putative "good-gene" benefits, even though the latter will be difficult to rule out empirically.

Nevertheless, first males might actively try to copulate with females in prospect to sire additional offspring in a potential subsequent clutch. The observation that females typically reject these copulation attempts (Schamel et al., 2004b) further speaks against females actively storing sperm from the first mate for "good gene" benefits, as they would be expected to copulate with their first mate as long as they can, if their strategy would be to fertilize offspring in the second clutch with sperm from the first mate. Nonetheless, we could show that first mates occasionally manage to sire extra-pair offspring in the second clutch of polyandrous females (Chapter 1), particularly if the second clutch was laid in quick succession from the first one. Furthermore, we observed some extra-pair copulations, which are another potential source of extra-pair offspring (Chapter 1). Taken together, female mate acquisition behaviour, extra-pair copulations and short intervals between clutches can result in genetic polyandry and in intense sperm competition in red phalaropes (Johnson and Briskie, 1999). Genetic polyandry in red phalaropes is consequently more strongly driven by direct reproductive gains in females (mate acquisition) and males (extra-pair paternity) and is not or to a lesser extent driven by female's cryptic mate choice.

The strongest potential for mate choice among the two polygamous species is expected in pectoral sandpiper females. Unfortunately, we are lacking knowledge of how pectoral sandpiper females choose males. Physical traits like size or courtship displays could be direct quality indicators or the size and quality of the territory (i.e. good nesting sites and food quantities) could be indirect measures of mate quality. In pectoral sandpipers, males arrive before females on the breeding grounds and females, like males, sample multiple breeding sites (Kempenaers and Valcu, 2017; Chapter 3). Local male densities could therefore also play a role in female site choice. However, causalities are difficult to disentangle as male tenure is also linked to female breeding densities (Kempenaers and Valcu, 2017). Detailed information on female movements between male territories and a quantification of male encounters, as well as, information on habitat choice for nesting sites are lacking, but would be essential to understand female mate choice in this species.

To summarize, in both species mate choice is expected to maximise reproductive success mainly directly rather than indirectly. In red phalaropes, mate choice could happen in males or females, depending on the operational sex ratio, which could allow direct choice between mates. In contrast, in pectoral sandpipers, sex roles in mate choice are fixed, with males unlikely choosing among different females and with females taking the role of the choosy sex. Yet, it remains to be studied how much physical traits and elaborate courtship displays, compared to the quality of the territory, play a role in female mate choice in pectoral sandpipers. Physical traits, like size or coloration could also play a role in mate choice in red phalaropes by both sexes, but as for pectoral sandpiper females, this remains to be investigated.

Environmental influence on polygamy, mate searching and mate choice

In the previous sections I gave a general overview of the mating system of red phalaropes and pectoral sandpipers, and I discussed the selective pressures and intersexual conflicts of sexual selection on the mating system of both species. In this section, I want to focus on how the environment influences the rates of polygamy, mate searching behaviour and mate choice, and on how these ultimately influence the strength of sexual selection in both species.

Artic summers are generally short and characterised by high between-year variation in snow accumulation and timing of snowmelt (Smith et al., 2010). For shorebirds, especially in the Arctic, it is important to match incubation and chick rearing with local food peaks (Saalfeld et al., 2019). In particular, uniparental care is only possible with enough easily accessible resources. Short seasons can therefore limit the possibilities for red phalarope females to become polyandrous or to lay replacement clutches for the same mate. We observed this in the first two years of our study on red phalaropes, in which snowmelt was particularly late for Utqiaġvik. This limited the total number of nesting attempts and the possibility for females to lay multiple clutches, including replacement clutches (Chapter 1). While part of the population still managed to breed, further north in the breeding range (i.e. Canadian Arctic, Svalbard and Taymyr Peninsula) this can even lead locally to no breeding attempts at all (Mayfield, 1978). However, as red phalaropes are non-territorial and mobile they might breed elsewhere and females might have one or multiple clutches in areas where the conditions are good early in the season or they might even move into areas with later food peaks and lay there too. On a small scale, this might explain the differences between the higher rates of polyandry reported by Schamel and Tracy (1977), which was conducted in the earliest habitat available in the area, compared to our study site where snow melts a bit later. In contrast, polyandrous species breeding in the temperate zone, such as the spotted sandpiper, have a much longer breeding season, allowing females to lay up to six clutches within a season, and, in this species, 33-100% of the females (N = 13 years) were found to be socially polyandrous (Oring et al., 1992; Oring et al., 1991). The length of the breeding season is therefore one main factor that can limit the possibility for polyandry in the red phalarope.

In the same way, pectoral sandpipers' females will start breeding later and locally in lower densities, if snowmelt is delayed. This will likely reduce the potential for males to sire offspring in general and even more so to sire offspring with multiple females. While this direct link still needs to be formally tested, there is a clear correlation with the local tenure, the time males stay locally, with the number of breeding females (Kempenaers and Valcu, 2017). This is further supported by the positive relationship of fertile females and resident males (Kempenaers and Valcu, 2017). In general, the longer the breeding season over the breeding range, the more asynchrony in laying exists. This can be beneficial for males that visit many sites and are locally competitive to sire offspring, which could create a stronger reproductive skew between males.

Another environmental factor that can increase the rates of polygamy is nest predation. If females replace lost clutches (assuming there is time to do so, see above), this gives phalarope females the chance to replace the clutch previously laid by a different female for this male. While this can generally increase social polyandry, we observed that around half of the replacement clutches are actually laid by the previous social partner of the male (5 of 11), indicating a preference of males to re-nest with the same female (Chapter 1). The same preference for previous social mates was found to limit the rates of social polyandry in red-necked phalaropes (Schamel et al., 2004a). Note that phalarope males can become polygynous in this way, but are not able to care for two successful clutches within one breeding season. In the same way, males of pectoral sandpipers can potentially sire more offspring when females re-nest. In general, overall reproductive success of individuals that breed in areas with high nest predation rates will be lower and the benefits of polygamy will be reduced in this way. Species that are not site faithful, like red phalaropes and pectoral sandpipers (Kwon et al., 2022), could therefore benefit from choosing sites with low potential of nest predation. Nest predation of shorebird nests in the Arctic is linked to lemming densities, as the Arctic fox *Vulpes lagopus* and jaegers *Stercorarius* spp. focus on lemmings when they are in high densities and they focus on shorebird nests, when lemmings are in low densities (McKinnon et al., 2014). However, whether lemming densities directly influence breeding site selection and how birds could assess potential predation risk remains unclear. Nevertheless, nest predation will influence the operational sex ratio, if the season is still long enough to replace the lost clutches.

The high spatiotemporal variation of habitat availability (due to variation in snow accumulation and in timing of snowmelt) combined with variation in predation rates (due to variation in lemming densities and predators), creates heterogeneity in breeding site quality for shorebirds over the Arctic. Red phalaropes and pectoral sandpipers are not site faithful or mate faithful between breeding seasons, and they could therefore benefit from searching for local optima within parts or within their whole breeding range. Finding one or multiple high-quality sites can then increase the potential to sire offspring with multiple mates for pectoral sandpiper males and red phalarope females. This breeding site sampling behaviour is therefore in part also mate searching behaviour, in particular for sites with female (pectoral sandpiper) or male (red phalarope) biases in the operational sex ratio.

As the quality of distant locations is likely unpredictable, pectoral sandpiper males seemingly search with nomadic movements for high quality sites (Kempenaers and Valcu, 2017). We were able to show in Chapter 3, that the prevailing wind conditions influence where pectoral sandpiper males fly. This shows how the search for breeding sites and ultimately mate searching behaviour is influenced by the cost of reaching different breeding sites. As pectoral sandpiper males arrive first on the breeding grounds (i.e. before females) and variation in wind conditions is particularly high in the Arctic (Proshutinsky et al., 2015), this could also lead to differences in local adult sex ratios. For example, if the later arriving females experience different wind conditions, this could make it more likely that they fly to different areas of the breeding range (assuming that females are influenced in breeding site sampling behaviour by the wind conditions in a similar way). Consequently, this could create higher potential for skewed sex ratios in pectoral sandpipers, compared to red phalaropes, which migrate usually in mixed flocks and in which the sexes arrive at the same time. After female pectoral sandpipers arrive, both sexes likely also fly in mixed flocks, which might reduce the potential of locally highly skewed sex ratios and therefore of variation in polygyny-potential for males.

Local differences in breeding site quality and local biases in the adult and operational sex ratio will consequently influence mate searching behaviour and therefore mate interaction rates, which will ultimately influence mate choice. In red phalaropes, as previously described (see “Pair bond vs. short association”), male or female biased operational sex ratios influence which sex is more competitive and more likely to initiate pair formation and also which sex invests more into mate guarding behaviour (Chapter 2). In this way, the limiting sex can be choosier. Further mate searching behaviour is then limited by the necessity for females to pair for some time with a male. In contrast, some pectoral sandpiper males can already sire offspring by only staying one day at a particular breeding site and can therefore benefit from visiting more sites compared to red phalarope females. Particularly in a situation with a long breeding season and high asynchrony of site quality over the breeding range, some pectoral sandpipers males could sire offspring at many different breeding sites.

Conclusion

This dissertation adds to a - ever since Darwin - growing number of studies on sexual selection. Despite the efforts in the field, though, a detailed description of the mating system is still missing for many species. This information would be particularly interesting when considering rare mating systems, since these “exceptions” are often providing special insights into selection pressures. Good-quality data is also important for meaningful meta-analysis, since small samples sizes and anecdotal data can potentially limit the power of their general conclusions. For example, an influential study on the evolution of sex roles in birds by Liker et al. (2013) used the adult sex ratio estimation for red phalaropes published by Whitfield (1995). This estimate is based on a small population in Iceland that consisted only of 8 birds in 1986 and 11 birds in 1987, which, as already mentioned before, might be too limited to represent this species. Considering that this species is one of the very few with this rare mating system, it seems pressing the need to gather new data that can supplement the already existing one. Nevertheless, Whitfield (1995) is still the only study providing an adult sex-ratio estimate for this species as of now, mostly because it is not trivial to estimate this population parameter when individuals are frequently moving between sites, as it is the case in Utqiaġvik. To estimate adult sex-ratios under such dynamic local conditions, one first needs to quantify sex specific differences in movements and detectability, which could be done with the help of animal tracking devices.

On top of this, our study proves that when descriptions are indeed available, further studies can still challenge the until-then valid information and add one more piece to what is actually a complex mosaic of knowledge rather than a still frame. In the case of our work, a “textbook example” of classical polyandry, the red phalarope, turned out to be less polyandrous (socially and genetically) than previously thought. However, while our study provides a big sample size for the rates of extra-pair paternity and the rates of social polyandry and re-nesting, it still only comprises three years of detailed data at one location of the breeding range. Further studies are necessary to understand local and breeding-range wide dynamics of social polyandry, renesting and extra-pair paternity in relation to spatiotemporal variation in habitat availability and local skews in sex ratios.

Furthermore, it would be fantastic if this study would be replicated in some years. Climate change is particularly influential in the Arctic and it will change the tundra in the next decades (Jansen et al., 2020). Red phalaropes and pectoral sandpipers have potentially a high ability to adapt to changes because of their opportunistic settlement strategy, which allows them to choose from multiple sites and to adjust to local conditions (Saalfeld and Lanctot, 2015, 2017). A longer breeding season could also provide potential for red phalarope females to lay multiple clutches and pectoral sandpiper males to sire more offspring. However, predictions are not linear and the recent exceptionally late springs in Utqiaġvik suggest that climate change might also create more inter-annual variation in local conditions, rather than a gradual advancement in snowmelt dates. This makes it difficult for shorebirds to match local food peaks and does not necessary increase productivity in their populations (McGuire et al., 2020; Meyer et al., 2021; Saalfeld et al., 2019; Weiser et al., 2018). With all publications in this study, we provide full data and code access, and detailed method descriptions to give the opportunity to future scientists to replicate them. In this way we hope to contribute to future studies, which can use our data for comparison.

Polyandry and polygyny are the mating systems which are characterised by either strong female-female or male-male competition. However, what came about while comparing red phalaropes and pectoral sandpipers is that both extremes are not two sides of the same coin. Females by definition lay eggs. This creates the need to associate for some time with males to provide some assurance of paternity, to "persuade" him to take care of the offspring alone. In contrast, males of highly polygynous species only provide sperm, and females, always certain about their maternity, will always take care of their offspring. Consequently, females in polyandrous species, invest more in the offspring than males in polygynous species. Intrasexual selection is strong in both mating systems, but usually considerably weaker in polyandrous species. This is due to the different time it takes for females or males to re-join the mating pool. While female red phalaropes potentially need at least five days, but in most cases over one week to lay a clutch for a male, male pectoral sandpipers only need one copulation and are only limited by their sperm production. Therefore, in situations with male-biased operational sex ratio, male-male competition can be stronger in red phalaropes than female-female competition. In contrast, female-female competition in pectoral sandpipers will likely never be stronger than male-male competition.

These dynamics of intrasexual competition also influence mate choice, which is expected to play a bigger role in the limiting sex, which has the possibility of simultaneous choice between more than one mate. Mate choice is potentially most relevant in female pectoral sandpipers that have the highest potential to locally profit from a high-quality male. They can benefit either directly, since males protect females from harassment by other males, or indirectly from “good gene” benefits. However, passive mate choice could also happen, as a consequence of habitat choice. Future studies on female movements and mate selection are necessary to disentangle causalities. Male pectoral sandpipers in contrast are not expected to be choosy at all. In red phalaropes, males are expected to be choosier than females, although this can change, depending on the operational sex ratio. From the female side it seems that direct fitness benefits, like finding a male to lay a second clutch for, are more important than indirect fitness benefits. Patterns of extra-pair paternity are therefore more likely simply explained by female strategies to gain additional mates, rather than through “good gene” benefits as previously suggested in sequentially polyandrous species (Dale et al., 1999; Oring et al., 1992).

In conclusion, intrasexual selection is apparently stronger in both species than intersexual selection, and direct fitness benefits more important than indirect fitness benefits in mate choice. Nevertheless, it is important to consider the interplay of both intra- and intersexual selection mechanisms. These dynamics can be situation dependent and are influenced by the spatiotemporal variation in environmental conditions and the operational sex ratio. Within this dynamic it is important to consider individual strategies that maximise reproductive success from the male and female perspective and also to consider evolutionary constraints. Recent advances in tracking technology and modern genetic methods combined with behaviour observations are one way to further enhance our knowledge of mating behaviours and sexual selection.

Outlook

A logic addition to this thesis is a more detailed look at red phalarope movements. For Chapter 2, we purely used the GPS positions to classify if breeding pairs were together, without consideration of were individuals went. In my next project, I want to use these fine-scale tracking data, which are limited by data downloaded locally, in combination with satellite telemetry data, which are downloaded remotely, to quantify the spatiotemporal distribution of individuals. Using data from known breeders as calibration, I will try to identify further potential nesting attempts of individuals that left the study area. Since red phalaropes are non-territorial and can move over large distances, females could have additional breeding attempts in faraway sites. Therefore, the rate of polyandry could potentially be higher than documented locally in this study. The quantifications of differences in movements and detectability between males and females could furthermore allow estimations of the local adult and operational sex ratio and potentially reveal drivers of local differences in them. Knowledge of the scale of individual movements and sex-specific differences in them, will further add information which will improve our understanding of the mating system of red phalaropes.

Further technological developments will hopefully in the near future allow scientists to combine fine-scale location data with accelerometery data in remotely downloadable small transmitters. This will allow scientists not only to track movements of individuals constantly, but additionally to classify their behaviour constantly, based on the accelerometery data (Couzin and Heins, 2023). This could allow for example to gather detailed data of behaviours throughout the whole breeding season and potentially multiple years. In the case of red phalaropes, for example, these data could show when females engage with other males with frequent copulations and show scraping behaviour, which can indicate a breeding attempt. Similarly, for pectoral sandpipers, such devices would allow scientists to follow individuals throughout their breeding range and to classify at which places they are territorial and to classify the frequency of courtship displays, maybe even copulations. This will give deeper insights into the potential reproductive success of individuals. Similarly, incubation patterns and chick rearing behaviour can give direct insights into the reproductive success of the caring sex (Picardi et al., 2020; Schreven et al., 2021). Ultimately, advanced technology will allow scientist to not only quantify behaviours and reproductive success of one breeding season, but over their whole life (Weimerskirch et al., 2014). This will allow to estimate the lifetime reproductive success of individuals of different species in the wild, which can be linked to their different life history strategies and will open the door for many new discoveries in behavioural ecology.

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Author contributions

Author contributions as published / submitted in the style of the corresponding journal.

Chapter 1

J.K., M.V. and B.K. conceptualized the project and the intensive study. R.L. and S.S. conducted fieldwork and contributed the long-term monitoring data. J.K., M.C., B.K, M.V. and S.K. were involved in the field work of the intensive study. M.C. and S.K. performed the molecular analysis. B.K. performed the parentage analysis. J.K. analysed the data with input from B.K., M.V and R.L. J.K. and B.K. wrote the paper with input from R.L. M.C. illustrated the red phalaropes used in the figures. All authors approved the final manuscript.

Chapter 2

J.K.: Conceptualization (equal); Methodology (lead); Validation (lead); Formal analysis (lead); Investigation (lead); Data curation (equal); Writing – original draft (lead); Visualization (lead); Project administration (equal). M.V.: Conceptualization (equal); Methodology (supporting); Validation (supporting); Formal analysis (supporting); Investigation (supporting); Data curation (equal); Visualization (supporting); Supervision (supporting); Project administration (supporting); Writing – review and editing (supporting). M.C.: Methodology (supporting); Investigation (supporting); Project administration (supporting); Writing – review and editing (supporting). W.F.: Conceptualization (supporting); Formal analysis (supporting); Writing – review and editing (supporting); Visualization (supporting). B.K.: Conceptualization (equal); Methodology (supporting); Formal analysis (supporting); Investigation (supporting); Resources (lead); Writing – original draft (supporting); Writing – review and editing (lead); Visualization (supporting); Supervision (lead); Project administration (equal), Funding acquisition (lead).

Chapter 3

B.K. and M.V. initiated the study; J.K. analysed the data with input from B.K. and M.V; all authors interpreted the results; J.K. and B.K. wrote the paper with input from M.V.

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Bart Kempenaers, Doktorvater Johannes Krietsch, Doktorand

Statutory declaration and statement

Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbständig und ohne unerlaubte Hilfe angefertigt worden ist.

Seewiesen, den 21.06.2023 ....

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Erklärung

Hiermit erkläre ich, dass die Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist. Des Weiteren erkläre ich, dass ich mich nicht anderweitig einer Doktorprüfung ohne Erfolg unterzogen habe oder ohne Erfolg versucht habe, eine Dissertation einzureichen oder mich einer Doktorprüfung zu unterziehen.

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