

Female choice of young sperm in a genetically monogamous bird

Richard H. Wagner^{1*}, Fabrice Helfenstein² and Etienne Danchin²

¹Konrad Lorenz Institute, Austrian Academy of Sciences, Savoyenstrasse 1a, A-1160 Vienna, Austria

²Universite Pierre et Marie Curie, Laboratoire d'Ecologie, CNRS-UMR 7625, Bâtiment A, 7 Etage, Case 237, 75252, Paris CEDEX 05, France

* Author for correspondence (r.wagner@klivv.oeaw.ac.at).

Recd 29.09.03; Accptd 13.11.03; Published online 21.01.04

When females copulate with multiple males the potential exists for female sperm choice. Females may increase the probability of being fertilized by preferred males by selectively retaining their sperm while ejecting the sperm of unfavoured males. An alternative criterion to male quality for female sperm choice may be sperm age because old sperm degrade and can lead to zygote death or unhealthy offspring. Here, we report that in a genetically monogamous bird, the black-legged kittiwake *Rissa tridactyla*, females eject their mates' sperm according to when the copulations were performed. Following copulations that were performed approximately two weeks before egg laying, females ejected inseminations at high frequencies while retaining inseminations that occurred soon before laying. Females that suffered hatching failure had ejected sperm from early copulations less than half as frequently as females whose entire clutches hatched. Furthermore, chicks that hatched from eggs fertilized by old sperm were in poor condition relative to those fertilized by young sperm. These findings support the 'young sperm' hypothesis, which predicts that females choose fresh sperm to avoid reproductive failure and are the first to show intra-male sperm choice by females.

Keywords: sperm choice; cryptic female choice; sperm competition; kittiwake; infertility

1. INTRODUCTION

Sperm competition may be viewed as male–male competition after copulation (Parker 1970). Recently, the other mode of sexual selection identified by Darwin, female mate choice, has been extended to the post-copulatory female choice of sperm from different males (Eberhard 1996; Olsson *et al.* 1996; Birkhead 1998; Pizzari & Birkhead 2000; Siva-Jothy 2000). A taxonomically widespread mechanism of female sperm choice is sperm ejection, which has been reported in insects, birds and mammals (reviewed in Eberhard 1996). Females may selectively eject the sperm of inferior males while retaining the sperm of preferred males (Eberhard 1996; Birkhead 1998) as has been experimentally shown (Pizzari & Birkhead 2000).

Although sexual selection has been the main focus of

female sperm choice, natural selection may also favour sperm choice because sperm degrade with age, potentially resulting in infertility or zygote death (Warren & Kilpatrick 1929; Lodge *et al.* 1971; Birkhead *et al.* 1993). Degraded sperm are also known to produce unhealthy offspring (Blount *et al.* 2001). Accordingly, females may attempt to ensure insemination with young sperm by choosing males that copulate frequently (Siva-Jothy 2000). Female sperm choice has thus far been examined exclusively in species in which females copulate with multiple males (Davies 1983; Eberhard 1996; Pizzari & Birkhead 2000), whereas studies of species in which most females copulate with only one male are lacking. Here, we examine the common occurrence of post-copulatory sperm ejection in a monogamous species, the black-legged kittiwake. Kittiwakes are sexually monomorphic, cliff-nesting gulls in which both sexes provide parental care (Cramp 1983). We never witnessed females seeking extra-pair copulations in tens of thousands of nest hours of observation, with the only two extra-pair copulations we observed apparently coerced by males (Helfenstein *et al.* 2004). These behavioural observations are consistent with paternity analyses, which revealed no evidence of extra-pair paternity in 119 offspring (Tirard *et al.* 2002; Helfenstein *et al.* 2004). The monogamous mating system of kittiwakes allows us to consider explanations of female sperm choice that may not involve sexual selection.

2. METHODS

The study was conducted in Cap Sizun in western France (48°5' N, 4°36' W), where more than 15 000 kittiwakes have been colour-ringed since 1979 (Helfenstein *et al.* 2003a). During ringing, nestlings were measured at an age of 8–25 days. We measured wing length within 1 mm with a stop-ended ruler and body mass within 5 g with a Pesola spring balance. Our methods for recording sperm ejection are detailed in Helfenstein *et al.* (2003b), but in summary, in 1999, 2000 and 2001 we recorded all copulations performed by 188 focal pairs comprising a combination of individuals that were observed in one or more years. Sexual behaviour was easy to observe on the open nesting ledges (figure 1a). Following the termination of each copulation we recorded whether and when females made a cloacal expulsion within 90 s of the male dismounting. Ejections following copulation comprised a forceful muscular contraction and the expulsion from the cloaca of white fluid, which often carried beyond the nesting cliff, landing in the sea below where it was impossible to collect. We assume, however, that females ejected sperm as well as possibly faeces because (i) as in other species these ejections occurred soon after copulation, presumably before sperm had time to travel up the female reproductive tract (Wagner 1991; Pizzari & Birkhead 2000), and (ii) for defecations performed in the absence of copulation, faeces were dropped without noticeable force. We confirmed that cloacal expulsions occurred non-randomly after copulation by quantifying the defecation rates of females. Females ($n = 138$) were 290% more likely to extrude a white substance from their cloaca within 90 s of finishing a copulation than at other times, a difference that was significant ($p = 0.014$) (Helfenstein *et al.* 2003b). For these reasons and because of the patterns that we report here we assume that sperm was ejected during post-copulatory expulsions.

The proportion of copulations followed by sperm ejection was highly repeatable within individual females among years (repeatability = 0.58, $F_{43,52} = 4.03$, $p < 0.0001$) (Lessells & Boag 1987). This high repeatability allowed us to average data for individuals that were observed in more than 1 year.

3. RESULTS

We recorded 634 copulations of which 142 (22.4%) were followed by sperm ejection that occurred a mean of 45.9 s (s.d. = 24.0, range of 1–90) after the last insemination. Females laid one to three eggs with a mean clutch size of 2.01 (s.d. = 0.35). No copulations were observed after the laying of the second egg. Post-copulatory sperm ejection (i.e. the proportion of copulations followed by

sperm ejection) declined markedly starting 16 days prior to the laying of the first egg (Spearman's rank correlation $r = -0.96$; figure 1*b*). The temporal decline in sperm ejection indicates that females tended to eject sperm from early copulations while retaining sperm from late copulations, as predicted by the young sperm hypothesis (Siva-Jothy 2000).

The young sperm hypothesis also predicts that the retention of old sperm results in zygote death (Lodge *et al.* 1971; Siva-Jothy 2000). We examined this prediction by comparing sperm ejection frequencies in relation to two factors: hatching failure and the timing of copulation (see figure 2). Out of the 188 focal nests, females produced 153 clutches in which 37 (24%) included at least one egg that failed to hatch. Figure 2 presents the means calculated from all 153 clutches and illustrates that in the early period, sperm ejection frequencies of females that did not suffer hatching failure were more than twice as high as for females that did suffer hatching failure (females without hatching failure, mean \pm s.d. proportion of copulations followed by sperm ejection = 0.354 ± 0.426 , $n = 97$; females without hatching failure = 0.151 ± 0.281 , $n = 32$ (figure 2*a*)). By contrast, sperm ejection was similar in the late period between females that did not and did suffer hatching failure (females without hatching failure = 0.161 ± 0.295 , $n = 103$; females with hatching failure = 0.141 ± 0.296 , $n = 35$ (figure 2*b*)).

In statistically examining hatching failure and sperm ejection frequency we used all of our data on hatching failure while avoiding using individual females more than once. For each female ($n = 100$), we calculated hatching failure as the number of hatched eggs divided by the total number of eggs laid by an individual female over the 3 years of the study, producing a 10-level variable. We averaged the sperm ejection frequency of each female in each period. Sample sizes differ between the two periods because some females were observed copulating in only one of the periods. We used a polytomous regression model (SAS GENMOD procedure) with multinomial distribution and a multilogit link function. Sperm ejection frequency was used as the explanatory variable and hatching success was used as the dependent variable. We found that sperm ejection frequency was significantly related to hatching success in the early period ($\chi^2 = 8.68$, d.f. = 1, $n = 78$, $p = 0.003$) but not in the late period ($\chi^2 = 0.84$, d.f. = 1, $n = 88$, $p = 0.36$). Using other cut-off dates to produce different early and late periods yielded similar results. These findings were unrelated to clutch size, which did not differ for females that did and did not suffer hatching failure (mean \pm s.d. clutch size of females without hatching failure: 2.02 ± 0.264 , $n = 58$; mean \pm s.d. clutch size of females with hatching failure: 2.03 ± 0.413 , $n = 20$; Mann-Whitney U -test: $z = 0.17$, $p = 0.86$).

We also examined whether the body condition of offspring was related to sperm age. We did this by correlating sperm ejection frequency and chick condition in the early and late periods. We used the residuals of the regression of chick body mass on wing length as a measure of chick condition. In broods with more than one chick we averaged the residuals, and for females observed in more than one year we averaged the values of each brood. Sperm ejection frequency was significantly correlated with mean chick condition in the early period (SAS General Linear

Model: $F_{1,67} = 4.42$, $p = 0.04$), but not in the late period ($F_{1,75} = 1.56$, $p = 0.22$), suggesting that fertilization by older sperm may have reduced chick condition. These results were unrelated to brood size, which did not covary with sperm ejection frequency either in the early period (Spearman's rank correlation: $r_s = 0.15$, $p = 0.21$, $n = 69$) or in the late period ($r_s = -0.06$, $p = 0.58$, $n = 77$). These results were also unrelated to chick age at the time of measurement, which was not significantly correlated with sperm ejection in the two periods (linear regression, early period: $F_{1,67} = 0.96$, $p = 0.33$; late period: $F_{1,75} = 2.66$, $p = 0.11$).

If sperm age is associated with offspring condition it may also be associated with offspring survival. Offspring survival was calculated as the proportion of chicks that fledged for a given female over the entire study period. Sperm ejection frequency was averaged for females observed in more than 1 year. We used a polytomous regression (multinomial distribution, multilogit link function) to test for a relationship between sperm ejection frequency in the two periods and fledging success. Sperm age was not related to chick mortality in either the early period ($\chi^2 = 1.90$, d.f. = 1, $n = 76$, $p = 0.17$) or the late period ($\chi^2 = 0.01$, d.f. = 1, $n = 83$, $p = 0.92$).

4. DISCUSSION

Female kittiwakes tended to eject their mates' sperm following seasonally early copulations while increasingly retaining sperm as egg laying approached. By ejecting sperm that would have been old by the time of fertilization, females appear to pursue a strategy of avoiding hatching failure caused by old sperm. An additional line of evidence that old sperm are deleterious is that sperm ejection was related to chick condition in the early but not the late period. This suggests that even when the retention of old sperm did not lead to hatching failure, eggs fertilized by old sperm may have produced offspring in an inferior condition. This finding is consistent with research in humans and domestic animals, which shows that degraded sperm adversely affect offspring health (Blount *et al.* 2001).

Hatching failure can be caused by embryo death, or alternatively, by infertility owing to a shortage of sperm (Birkhead *et al.* 1995). Infertility is unlikely to explain hatching failure in kittiwakes because females that suffered hatching failure ejected sperm less frequently than females that did not. Furthermore, infertility cannot explain the relationship between sperm ejection and chick condition.

We did not find a link between the retention of old sperm and chick mortality. Although chicks in poorer condition might be expected to suffer higher mortality, other common causes of mortality we have observed such as predation, parasite infection and siblicide may outweigh any possible negative effects of old sperm. Nevertheless, it would be interesting to examine over the long term whether offspring that were produced from old sperm suffer shorter lifespans or other fitness consequences.

Our findings raise the question of why females accept copulations from their mates and then eject their sperm. A possible explanation is that by accepting copulations, females induce their mates to produce fresh sperm that are less likely to result in embryo death (Siva-Jothy 2000) or chicks in poor condition. Another explanation may be that females can reduce conflicts with their mates by

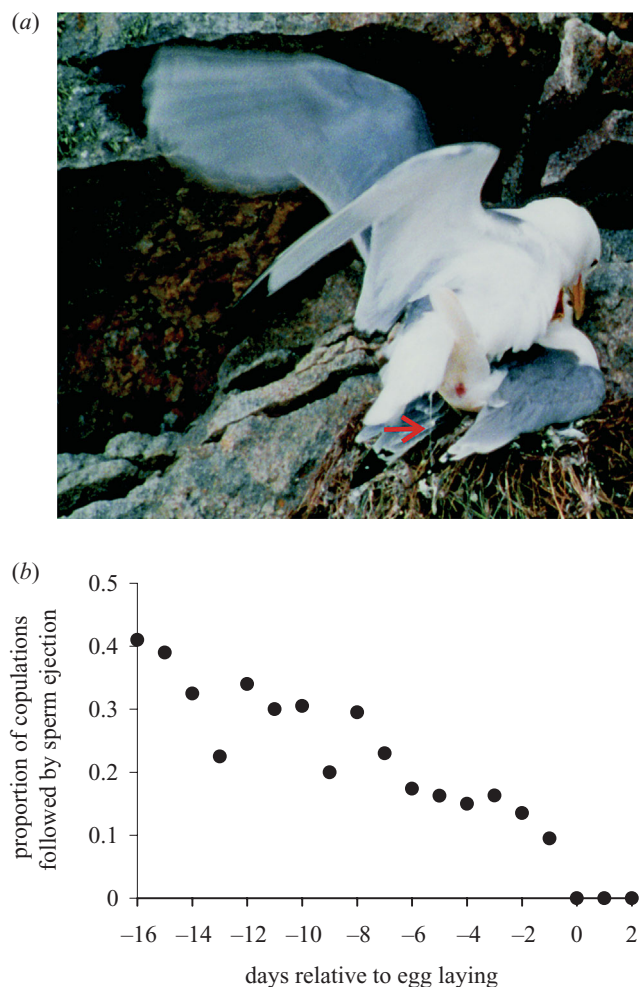


Figure 1. (a) A pair of black-legged kittiwakes copulating on their nest. The female's cloaca is open to receive sperm and, as indicated by the red arrow, a thin thread of semen can be seen dangling from the corner of the male's tail. (Photograph by Yannick Coupry.) (b) The decline in sperm ejection with time. Females decreasingly ejected their mates' sperm as egg laying approached ($r_s = -0.96$). Day 0 is the day on which the first egg was laid. Each point represents the proportion of copulations followed by sperm ejection on each relative day and was calculated from a mean of 37.5 (s.d. = 18.7) observations. In testing for the significance of this relationship, to avoid pseudoreplication we determined whether there was a positive or negative temporal change in sperm ejection for each female. For females observed copulating on 2 days we used the difference in the proportion of copulations followed by sperm ejection between the first and second day. For females observed on 3 or more days, we performed a correlation and used the sign of the coefficient. Out of 54 females that were observed copulating with their mates on at least 2 days, 38 had decreased their ejection of sperm, 11 increased it and five showed no change. When we conservatively combined females that increased sperm ejection with those with no change, females were significantly more likely to have decreased sperm ejection (binomial test, two-tailed: $z = 2.99$, $p = 0.0028$).

allowing them to copulate, thereby increasing the male's confidence of paternity against occasional forced extra-pair copulations (Helfenstein *et al.* 2003b). This explanation is supported by evidence that females attempted to prevent their mates from witnessing the ejection of their

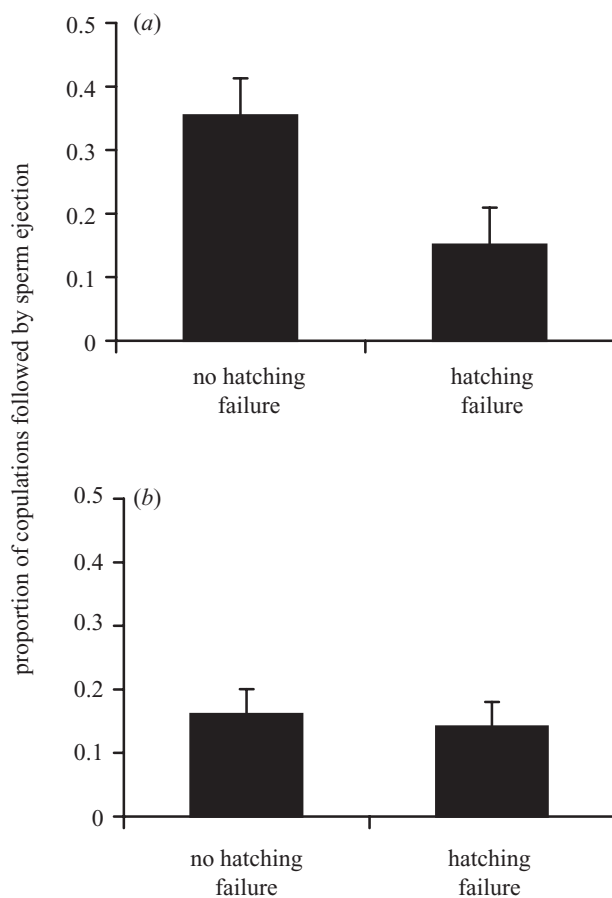


Figure 2. Sperm ejection and hatching failure. We divided the period of day -16 to day 2 in half (see figure 1), producing an early period (day -16 to -8) and a late period (-7 to 2). Females that suffered hatching failure were those that produced at least one egg that did not hatch. Hatching failure was associated with the retention of old sperm (a) but not with the retention of young sperm (b).

sperm by often delaying ejection until after the male had departed (Helfenstein *et al.* 2003b).

Another question raised by our findings is whether females simply ejected sperm because they were unable to store the amount of ejaculate transferred by their mates. We previously found no evidence for three predictions of the 'overflow' hypothesis (Helfenstein *et al.* 2003b) and report here that there was no relationship between mean copulation frequency and mean sperm ejection frequency ($r^2 = 0.007$, $F_{1,98} = 0.66$, $p = 0.42$). We also note that this possibility could not explain the links between sperm retention and hatching failure and sperm retention and chick condition.

Prior to this study, sperm ejection had been viewed exclusively as a mechanism of sexual selection that can allow females to choose sperm among males (Eberhard 1996; Pizzari & Birkhead 2000). However, the monogamous mating system of kittiwakes allows us to exclude sexual selection as an explanation of female sperm choice in this species. If sperm ejection were a mechanism used by females to select among the sperm of different males we would expect females to seek extra-pair copulations and to obtain extra-pair paternity, which was not the case (Helfenstein *et al.* 2004). If females were attempting to maximize their probability of being fertilized by extra-pair

males we would expect them to expel their mates' sperm with increasing frequency as they approached their period of peak fertility soon before egg laying (Colegrave *et al.* 1995), whereas they did the opposite (figure 1*b*).

As our results suggest, female kittiwakes can influence the age of their mates' sperm that they retain for fertilization. Whereas in promiscuous species, inter-male sperm choice is clearly sexually selected (Pizzari & Birkhead 2000), in monogamous kittiwakes intra-male sperm choice is apparently naturally selected. It has been proposed, however, that females of promiscuous species can obtain both benefits simultaneously: if attractive males copulate frequently then females can choose both sperm from high-quality males and young sperm (Siva-Jothy 2000). The behaviour of monogamous kittiwakes suggests that natural selection alone can favour female sperm choice in some species.

Acknowledgements

We thank Tim Birkhead, Donald Blomqvist, Dustin Penn, Tom Pizzari and Joel White for constructive comments.

- Birkhead, T. R. 1998 Cryptic female choice: criteria for establishing female sperm choice. *Evolution* **52**, 1212–1218.
- Birkhead, T. R., Møller, A. P. & Sutherland, W. J. 1993 Why do females make it so difficult for males to fertilize their eggs? *J. Theor. Biol.* **161**, 51–60.
- Birkhead, T. R., Veiga, J. P. & Fletcher, F. 1995 Sperm competition and unhatched eggs in the house sparrow. *J. Avian Biol.* **26**, 343–345.
- Blount, J. D., Møller, A. P. & Houston, D. C. 2001 Antioxidants, showy males and sperm quality. *Ecol. Lett.* **4**, 393–396.
- Colegrave, N., Birkhead, T. R. & Lessells, C. M. 1995 Sperm

- precedence in zebra finches does not require special mechanisms of sperm competition. *Proc. R. Soc. Lond. B* **259**, 223–228.
- Cramp, S. (ed.) 1983 *Handbook of the birds of Europe, the Middle East and North Africa.*, vol IV. Oxford University Press.
- Davies, N. B. 1983 Polyandry, cloaca-pecking and sperm competition in dunlocks. *Nature* **302**, 334–336.
- Eberhard, W. G. 1996 *Female control: sexual selection by cryptic female choice*. Princeton University Press.
- Helfenstein, F., Wagner, R. H., Danchin, E. & Rossi, J.-M. 2003*a* Functions of courtship feeding in black-legged kittiwakes: natural and sexual selection. *Anim. Behav.* **65**, 1027–1033. (DOI 10.1006/anbe.2002.2129.)
- Helfenstein, F., Wagner, R. H. & Danchin, E. 2003*b* Sexual conflict over sperm ejection in monogamous pairs of kittiwakes *Rissa tridactyla*. *Behav. Ecol. Sociobiol.* **54**, 370–376. (DOI 10.1007/s00265-003-0636-x.)
- Helfenstein, F., Tirard, C., Danchin, E. & Wagner, R. H. 2004 Low frequency of extra-pair paternity and high frequency of adoption in black-legged kittiwakes. *Condor*. (In the press.)
- Lessells, C. M. & Boag, P. T. 1987 Unrepeatable repeatability: a common mistake. *Auk* **104**, 116–121.
- Lodge, J. R., Fehchheimer, N. S. & Jaap, R. G. 1971 The relationship of *in vivo* sperm storage interval to fertility and embryonic survival in the chicken. *Biol. Reprod.* **5**, 252–257.
- Olsson, M., Shine, R., Madsen, T., Gullberg, A. & Tegelstrom, H. 1996 Sperm selection by females. *Nature* **383**, 585.
- Parker, G. A. 1970 Sperm competition and its evolutionary consequences in insects. *Biol. Rev. Camb. Phil. Soc.* **45**, 525–567.
- Pizzari, T. & Birkhead, T. R. 2000 Female feral fowl eject sperm of subordinate males. *Nature* **405**, 787–789.
- Siva-Jothy, M. T. 2000 The young sperm gambit. *Ecol. Lett.* **3**, 172–174.
- Tirard, C., Helfenstein, F. & Danchin, E. 2002 Polymorphic microsatellites in the black-legged kittiwake *Rissa tridactyla*. *Mol. Ecol. Notes* **2**, 431–433.
- Wagner, R. H. 1991 Evidence that female razorbills control extra-pair copulations. *Behaviour* **118**, 157–169.
- Warren, D. C. & Kilpatrick, L. 1929 Fertilization in the domestic fowl. *Poultry Sci.* **8**, 237–256.