SHORT COMMUNICATIONS

The Condor 98:410–413

SPERM TRANSFER IN THE ADÉLIE PENGUIN[1]

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Key words: Sperm transfer; sperm; fertility; copulation; copulation frequency; Adélie Penguin.

Sperm competition theory suggests that for a female that copulates with more than one male, the relative numbers of sperm inseminated will be important in determining which male fathers her offspring. Many studies of birds have reported insemination frequencies based on observations of apparent cloacal contact between male and female. Here we question whether cloacal contact is a reliable indicator of sperm transfer. This question has already been addressed in a small number of studies of captive species (Domestic fowl, *Gallus domesticus:* Penquite et al. 1930; Zebra Finch, *Taeniopygia guttata:* Birkhead et al. 1989; Bengalese Finch, *Lonchura striata:* Birkhead 1991). However Birkhead et al. (1988a) found differences in copulation behavior between wild and captive Zebra Finches, with wild birds copulating at a higher frequency than captive ones. So, although it has been shown for the above captive species that not all behaviorally successful copulations result in sperm transfer, it has yet to be shown for any wild species. Further, in these studies of captive species it was not determined whether sperm transfer failed as a result of the male's failure to ejaculate or absence of sperm in a successfully transferred ejaculate. Here we look at sperm transfer in a wild population of Adélie Penguins (*Pygoscelis adeliae*) by combining behavioral observations of cloacal contact and ejaculate transfer with collection of ejaculates and identification of presence or absence of sperm by microscopic examination. The aim of the study is to determine the true success rate, in terms of sperm transfer, of behaviorally successful copulations and the reason for failure of sperm transfer.

METHODS

The study was carried out at the Northern Rookery at Cape Bird, Ross Island, Antarctica (77°13′S, 166°28′E). The Adélie Penguins in this colony breed in distinct sub-groups of 50–1,000 pairs. Frequencies of cloacal contact and ejaculate transfer are based on observations of copulation behavior of 47 breeding females, made at a study site within the Northern Rookery (Hunter et al. 1995). The birds were observed for 16 hours a day during the period 30 October to 19 November 1993 and all copulation attempts were recorded. Observations were carried out during 65% of the potential time available. This proportion of time observed was used to estimate copulation frequencies per clutch from copulation frequencies observed. Mean frequencies of copulation per clutch are given ± standard deviation.

Copulation occurred when a male mounted a female and moved backwards along the female's back, beating his tail from side to side, moving it into position to one side of the female's upright tail and bringing his cloaca into line with the female's. The mean time from mounting to cloacal contact was 56.6 seconds (±14.5, $n = 11$ females) and cloacal contact lasted 1.9 seconds (±0.4, $n = 46$ females). After cloacal contact the male dismounted while the female remained still with her tail raised allowing an observer to make a detailed assessment of the presence or absence and position of any ejaculate. At this time, the outer surface of the female's cloaca contracted rhythmically, drawing any ejaculate inside. An ejaculate that had missed the center of the female's cloaca and had been deposited on the outer edge was often seen to be drawn across the outer surface of the cloaca towards the center and thence inside the cloaca (Hunter et al. 1995).

Each copulation attempt involving cloacal contact was categorised as follows; (1) cloacal contact with an ejaculate seen to be drawn into the center of the female's cloaca, (2) cloacal contact in which an ejaculate was seen to have missed the female's cloaca and was not drawn in, and (3) cloacal contact with no ejaculate seen.

In order to determine whether cloacal contact resulted in sperm transfer the cloacal contents of females engaging in copulation were collected. Random groups of ca. 300 pairs were observed from 14:00 hr to 16:00 hr on seven days during the period 10–20 November

[1] Received 11 April 1995. Accepted 20 October 1995.

1993 and at variable times of the day on five days during the period 13–19 November 1994. Each copulation attempt involving cloacal contact was categorised as above. Immediately following copulation the female of the pair was caught using a one-meter diameter hand net. The female's cloaca was manually everted and a microscope slide was gently pressed onto the inner surface of the cloaca 10 times. The slide was then air dried and the female was released. In all cases the female returned to her breeding site and partner after release. The time from cloacal contact to completion of the cloacal smear was estimated to be no longer than 90 sec for any individual. The slides were later stained with fluorescent Hoechst dye 33342 which caused the sperm nuclei to fluoresce (Wishart 1987), and 30 fields of view of each slide were examined under a microscope at 25× magnification. The presence or absence of sperm on each slide was recorded. The slides on which sperm were found, were further categorised into those with abundant sperm and those with only traces of sperm (a total of fewer than 10 sperm in 30 fields of view examined). Following cloacal contact cloacal samples were collected from 23 (11 in 1993; 12 in 1994) females into which an ejaculate was seen to enter, 18 (8; 10) females with a missed ejaculate and 21 (9; 12) females with no ejaculate seen. In addition cloacal samples were collected from 21 (11; 10) control females that were known not to have copulated for at least 30 minutes prior to capture.

In order to assess the possibility of some eggs being infertile, egg development was examined. An opportunistic collection was made of 36 eggs, abandoned by their parents during the chick-rearing period, after the presumed time of hatching, throughout the large Northern rookery at Cape Bird. Eggs abandoned at this time would have been incubated for more than three weeks (last egg in study group was laid on 9 December, first abandoned eggs collected on 2 January) and so would be expected to show signs of development. These eggs were opened and examined for visible signs of embryo development. Eggs laid in the study site were monitored by daily observations and egg loss and hatching were recorded.

RESULTS AND DISCUSSION

Adélie Penguins copulating with only one partner achieved 34.4 (±11.9, $n = 35$) cloacal contacts per clutch. However, only 20.0 (±8.8, $n = 35$) copulations per clutch involved observed ejaculate transfer. In 3.4 (±4.5, $n = 35$) copulations per clutch ejaculates were seen but missed the females cloaca and in 4.4 (±4.0, $n = 35$) copulations per clutch cloacal contact occurred but no ejaculates were seen. In the remaining 6.6 (±3.5, $n = 35$) copulations in which cloacal contact occurred, ejaculate transfer was undetermined. In other words only 58.8% (±16.5, $n = 35$) of copulations involving cloacal contacts resulted in successful ejaculate transfer. In 12.5% (±10.3, $n = 35$) of cloacal contacts no ejaculate was seen, in 8.8% (±9.4, $n = 35$) ejaculates missed the cloaca and in 19.9% (±9.4, $n = 35$) the whereabouts of the ejaculate was undetermined.

Presence and absence of sperm in cloacal samples from females in the four groups (ejaculate, missed ejaculate, non-ejaculate and control) are summarised in Table 1. In all but one of the 23 cases where an ejaculate was seen to enter the female's cloaca, large numbers of sperm were found in the cloacal smear. In the remaining case no sperm were found, even after extensive searching of the smeared slide. This pattern of presence or absence of sperm in cloacal smears was significantly different to that of females whose partners achieved cloacal contact with no ejaculate observed (Fisher Exact $P = <0.001$).

TABLE 1. Presence and absence of sperm in cloacal smears from females seen to have been inseminated, females that attained cloacal contact following which an ejaculate was seen to have missed her cloaca, females that attained cloacal contact with no ejaculate observed and control females that had not copulated within 30 minutes of cloacal sampling.

	Sperm			
	Present			
	Abundant	Traces*	Absent	Total
Cloacal contact with insemination	22	0	1	23
Cloacal contact with missed ejaculate	0	5	13	18
Cloacal contact with no ejaculate seen	1	6	14	21
No copulation	0	2	19	21

* Traces of sperm only (<10 in 30 fields of view).

Of 21 females involved in cloacal contacts during which no ejaculate was seen to be transferred to the female's cloaca, 14 had no sperm in the cloacal smear, six had traces of sperm and one had relatively large numbers of sperm present, though many of these sperm appeared to be broken. Comparing presence and absence of sperm, this was not significantly different to the control slides taken from females that had not copulated in at least 30 minutes (Fisher Exact $P = 0.055$). Of 18 females whose partners ejaculated but where the ejaculate was seen to have missed the female's cloaca, 13 had no sperm in the cloacal smear while five had traces of sperm. Again comparing presence and absence of sperm, this was no different to the control slides (Fisher Exact $P = 0.117$).

In their study of Turkeys (*Meleagris gallopavo*), Brillard and Bakst (1990) showed that it can take up to 48 hours for sperm to reach the site of sperm storage and that only 10% of the sperm inseminated reached that far, the rest were lost through the cloaca. Similarly Birkhead et al. (1993) found traces of sperm in the vaginas of female Zebra Finches 24 hours after insemination and suggested that these were some of the many sperm inseminated that never reached the female's sperm storage sites and were subsequently ejected from the female's cloaca (Howarth 1971, Birkhead et al. 1993). It seems likely that this is the explanation for the traces of sperm found in the missed ejaculate, non-ejaculate and control groups in the present study. This is supported by the observation that on the five slides from the 1994 breeding season on which traces of sperm were found, many of the sperm were broken.

In one copulation an ejaculate was seen to be trans-

ferred but no sperm were found in the cloacal smear (Table 1). There are two possible explanations for this; 1) sperm was transferred by the male but was all taken up into the female's reproductive tract before the cloacal smear was made. This seems unlikely given the short period of time within which the cloacal smears were taken and the presence of ejaculatory fluid in the cloaca at the time of smearing. 2) the copulating male was infertile and produced no sperm. In order to assess the possibility of some males being infertile, egg development was examined. Of 36 unhatched eggs, 77.8% (28) showed no sign of embryo development, while the remaining eight eggs contained embryos of varying sizes. In all, 159 eggs were laid in the study site in 1994. Of the 113 (71.1%) eggs that survived to the time of hatching, 88.5% (100) hatched while the remaining 11.5% (13) remained unhatched after the full incubation period had elapsed. With 11.5% (13/113) of surviving eggs failing to hatch and 77.8% (28/36) of these being undeveloped, it appears that 8.9% of Adélie eggs surviving the full incubation period, did not develop. Male infertility seems the most likely reason for eggs failing to develop, though alternative explanations, such as failure of the germinal disc to respond to fertilisation by the sperm or early embryo mortality cannot be ruled out (Romanoff 1960, Birkhead et al. in press). So it would seem that the most likely explanation for the lack of sperm in a small proportion of ejaculates is male infertility rather than rapid uptake of sperm.

It appears that in Adélie penguins, observation of cloacal contact alone is not a reliable indicator of insemination, and that observation of an ejaculate entering the female's cloaca is a better measure of insemination success. Observation of Adélie penguin insemination is possible because the female remains motionless with her cloaca clearly visible after copulation. In the majority of studies cloacal contact has been taken to indicate successful copulation as insemination cannot be observed directly (e.g., Hatch 1987, Møller 1987, Birkhead and Lessells 1988, Hatchwell 1988, Wagner 1991, Venier and Robertson 1991, Hunter et al. 1992, Schulze-Hagen et al. 1995). As reported above, female Adélie penguins copulating with one partner were inseminated 20.0 times per clutch. If cloacal contact had been taken as evidence of insemination, the frequency of inseminations per female would have been reported as 34.4 per clutch, substantially greater than the true frequency of inseminations.

Looking more closely at the failure of sperm transfer during behaviorally successful copulations, the mean percentage of these copulations that resulted in ejaculate transfer was 73.3% ($\pm$18.2, $n = 35$, range 25.0%–100%). Sperm were present in 95.7% (22/23) of inseminated ejaculates, so it appears that for the Adélie penguin unsuccessful sperm transfer during a behaviorally successful copulation resulted mostly from 1) failure of males to ejaculate and 2) incorrect positioning of the ejaculate on the female's cloaca resulting in failure of sperm uptake into the female's reproductive tract. In the latter case imprecise positioning of the ejaculate may have resulted from the actions of either the male or the female. In addition, a small proportion of behaviorally successful copulations failed because sperm was absent from a successfully positioned ejaculate. The mean percentage of behaviorally successful copulations that resulted in sperm transfer (70.1%) was similar to that found in studies of captive birds. In the Zebra Finch 63.6% of copulations with cloacal contact resulted in sperm transfer and in the Bengalese Finch 68.3% involved sperm transfer (Birkhead et al. 1988b, Birkhead 1991). It is not known whether failure of sperm transfer in these species is due to ejaculation failure or absence of sperm from ejaculates.

It is in a male's interest to copulate frequently with his partner in order to increase his chances of fertilising his partner's eggs (see Birkhead and Møller 1992) so it would appear maladaptive for a male to achieve a behaviorally successful copulation and then not transfer any sperm. There are a number of possible explanations for this paradox. (1) the male may be unable to transfer an ejaculate as a result of sperm depletion or depletion of ejaculatory fluids (Dewsbury 1982, Birkhead 1991). Adélie penguins have a high rate of copulations and so males may indeed run out of ejaculate material (Hunter et al. 1995). (2) males may allocate their ejaculates prudently. If ejaculates are limited, it may be in a male's interest to retain some ejaculates to use either in extra-pair copulations, or as retaliatory copulations in the event of his partner being involved in an extra-pair copulation (Birkhead and Møller 1992). (3) females may be able to control whether a male ejaculates or not (Birkhead and Møller 1993). The first two explanations assume male control of sperm transfer while the third assumes female control. Whatever the reason for males not transferring sperm, it is clear that in a substantial proportion of copulations no sperm is delivered. The question then arises—why males go through the motions of copulating with their partners without transferring sperm? Various benefits to copulating without sperm being transferred have been proposed. These include pair formation, pair bond maintenance, stimulation of reproduction and mate assessment (Chardine 1987, Fitch and Shugart 1984, Birkhead and Møller, 1992, Dewsbury 1983, Eberhard 1985, Westneat et al. 1990, Hunter et al. 1993).

In conclusion, it is clear that for the Adélie penguin cloacal contact is not a good indicator of sperm transfer and that most behaviorally successful copulations that fail to result in sperm transfer are due to the male failing either to produce an ejaculate or to accurately position the ejaculate on the female's cloaca.

We are very grateful to the New Zealand Antarctic Programme for providing logistical support during fieldwork and to Rob Harcourt and Marj Wright for field assistance. We thank Tim Birkhead for the use of his microscope and Jayne Pellatt and Bobbie Fletcher for technical advice. Also thanks to Tim Birkhead and Ken Wilson for helpful comments on the manuscript. The research was funded by the Natural Environment Research Council (FMH) and the University of Otago (LSD and GDM).

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