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Copulation behaviour in the Linnet *Carduelis cannabina* and the insemination window hypothesis

Jan Drachmann, Jan Komdeur and Jacobus J. Boomsma

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Copulation behaviour in the Linnet was investigated by focal observations of breeding pairs near their nest sites. Of 287 observed male copulation attempts only 37% resulted in cloacal contact. These successful copulation attempts were always preceded by female solicitation, indicating that the outcome of copulations was under female control. Relative to the day of first egg (day 0), males attempted copulations from day –8 to +9 with a peak of 2.4 copulation attempts per hour at day –4. However, females solicited copulations only from day –6 to +3, with a peak of 1.7 solicitations per hour on day –3. During the laying period male Linnets showed a significant peak in copulation attempts in the second hour after egg laying. The importance of this peak in copulatory behaviour after laying is discussed in relation to the insemination window hypothesis.

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In recent years a large number of studies has shown that socially monogamous bird species often engage in extra-pair copulations (reviewed in Birkhead and Møller 1992). To ensure the paternity of young in their own nest, males have evolved various behaviours to protect themselves against being cuckolded. The two most commonly used paternity guards are mate guarding, where males follow their mates closely, and frequent within-pair copulations (Birkhead and Møller 1992).

Female birds are capable of storing sperm for several days or weeks following copulation (Birkhead and Møller 1992). The fertile period, i.e. the period over which copulations can potentially result in fertilization, is therefore protracted. In domestic birds, ovulation occurs within two hours of the laying of an egg. The ovum must be fertilized within 30 min of ovulation before the perivitelline layer and albumen are deposited around the yolk, which effectively prevents sperm from reaching the ovum (Howarth 1974). During the laying period the oviduct contains a developing egg which

either inhibits or blocks the passage of sperm through the oviduct. However, soon after egg laying when the oviduct is not obstructed by the presence of an egg, some sperm can reach the site of fertilization in the infundibulum within 15 min of copulation (Howarth 1971). This short time period has been coined the insemination window (IW) by Cheng et al. (1983). By performing sperm competition experiments using artificial insemination and genetic markers in Mallards *Anas platyrhynchos*, Cheng et al. (1983) have shown that copulations during the IW are capable of fertilizing the next day's egg. This study has been influential and subsequent workers have assumed that the IW is a period of female peak fertility. However, in a recent paper Birkhead et al. (1996) show that the IW is not a period of peak fertility in females, but a time of relative inefficient use of sperm, since uptake of sperm by the female is reduced before and after oviposition.

Here we describe the copulation behaviour in the Linnet *Carduelis cannabina*, a sexually dichromatic and socially monogamous passerine, and show that male

Linnets copulate at a significantly increased rate in the second hour after egg laying. This peak in male copulation frequency during the laying period is discussed in relation to the IW.

Methods

Study areas and species

The study was performed in Denmark during the summers of 1994 and 1995 at Sjellemosegaard (56° 10' N, 9° 50' E) and in 1996 at Langholm (56° 30' N, 10° 50' E). The vegetation at Sjellemosegaard consisted of 13 ha of young Caucasian fir *Abies nordmanniana*, while the habitat at Langholm consisted of 5 ha of young cypress *Chamaecyparis lawsoniana* and common spruce *Picea abies* trees. Both areas were commercial plantations. The change of study area in 1996 was due to a very high predation level at Sjellemosegaard in 1994 and 1995, which impeded other studies conducted on reproductive behaviour of Linnets. There was no difference in Linnets' copulation behaviour between the two study areas.

On the basis of the number of nests found and the number of singing males recorded, the local breeding populations of Linnets were estimated to be 40–50 pairs at Sjellemosegaard and approximately 30 pairs at Langholm. Nests were built solely by the females, and often placed close to the nests of conspecifics, resulting in a semi-colonial breeding pattern. Breeding in the two populations was highly asynchronous, due to predation resulting in continuous renesting, and because the Linnets had multiple broods during the breeding season (April–August). Females laid one egg each day during the laying period and usually started incubating on the penultimate egg. Mean (\pm s.e.) clutch sizes were 5.0 ± 0.1 and 5.1 ± 0.1 eggs for Sjellemosegaard ($n = 47$) and Langholm ($n = 34$), respectively.

Behavioural observations

Behavioural observations were conducted between 0600 and 1200 hours from late April to mid July, and consisted of 30-min focal watches of individual pairs. Focal individuals were chosen among birds at any stage of breeding, from nest site selection to incubation. In most cases observations of a focal pair were conducted daily until the female commenced incubation. During the incubation period observations were made less frequently.

Male Linnets displayed carotenoid pigmentation on breast and crown, which appeared to be highly variable among individual males both in intensity and pattern. This variation allowed easy recognition of the focal males in our study population. Focal females were

more difficult to identify on their plumage coloration alone. However, females could easily be identified by their behaviour, because only the pair-females were engaged in nest building and incubation of eggs. So, since the pair-female usually visited the nest whenever she arrived near the nest site, and because she was followed closely by the guarding pair-male (Drachmann 1997), focal females could easily be identified correctly. In addition, two males and three females from five different focal pairs were colour-ringed, and could therefore be identified individually.

The copulation behaviour of focal birds was recorded continuously during the 30-min focal watches. An attempted copulation was defined as having occurred whenever the pair-male hovered over the back of the female. Whether a copulation was successful (presumed cloacal contact), and whether or not females solicited copulations were also recorded. The duration of copulations was measured with a stopwatch as the duration of mounting plus cloacal contact, i.e. not just cloacal contact.

Linnets are non-territorial and show no philopatry prior to breeding (Newton 1972). Focal individuals could thus only be followed after the onset of nest-building. Focal birds often exploited areas at a considerable distance from the nest site for foraging and collection of nest material. Therefore, each watch was restricted to 30 min of observation at the nest site, regardless of whether the birds were present or not. Thus the data presented here reflect the copulation behaviour of Linnets when they were near their breeding site.

Behavioural observations were related to the fertility of the female by making daily observations of nest-building behaviour, and by checking nests daily to determine when the first egg of the clutch was laid. To standardize the timing of the fertile period of females, the laying day of the first egg was designated as day 0.

Data analyses

Unless specified otherwise, copulation frequencies were calculated as copulations per hour of total time the focal nests were observed. Two measures of copulation frequency were calculated: The number of male copulation attempts per hour and the number of female-solicited copulations per hour.

With a mean clutch size of five, ovulation and fertilization of the eggs took on average place from day -1 to day $+3$, the ovulation period. However, an IW would only be operating from days 0 to $+3$, because on day -1 no prior egg was present in the female reproductive tract. Therefore, to facilitate testing of the IW hypothesis the nesting cycles of the females were divided into three periods according to the time of a possible IW: The pre-IW period (up to and including

day -1), the IW period (days 0 to +3), and the post-IW period (from day +4 onwards). To investigate whether focal pairs timed their copulations to coincide with the IW, the timing of copulations in the pre-IW period was compared with their timing in the IW period (during which time the female reproductive tract would have contained an egg). In this analysis the observations in the pre-IW and the IW period were sub-divided by the hour.

A total of 486 focal watches of 23 breeding pairs was conducted in 1994, 56 focal watches of two pairs in 1995, and 48 focal watches of two pairs in 1996. Unfortunately, not all focal pairs observed during the three years could be used in the analyses. Some females abandoned their nests and other nests were destroyed by predators before the first egg was recorded. The sample size was further reduced because in some focal pairs not all copulation attempts could be recorded with certainty. To obtain mean values only focal pairs that had been present for at least three focal watches on a given day were included in the analyses. These restrictions imply that sample sizes sometimes vary between analyses; consequently the relevant sample sizes are given in each analysis and figure. All focal pairs included in the analyses were considered to be statistically independent observations.

Nonparametric statistics (χ^2 -Friedman tests and r_s -spearman rank correlations) were used for all of the analyses in accordance with Zar (1984), since data were not normally distributed and could not be normalized with simple transformations. Results are presented as means \pm s.e. If sample sizes in figures are greater than one and no standard errors are given, then s.e. = 0.

Results

Copulations were usually associated with female nest visits, and 83% of 287 observed copulations took place immediately after the female had left the nest, sitting either in the nest tree or in the top of a nearby tree. Ninety-five percent of all observed copulation attempts took place within 10 m from the nest and 19% were performed in the nest tree. Only two copulations (<1%) were observed more than 20 m from the nest (35 and 40 m respectively). The males were usually nearby whenever the female left the nest, since the males were guarding their females by following them closely from the onset of nest-building until incubation (Drachmann 1997). When the female left the nest, the guarding male often attempted to copulate, but usually he was rejected. Only 37% of the 287 observed male copulation attempts were successful (i.e. apparent cloacal contact), and these successful copulations were always preceded by female solicitation. The female solicited by tilting forward, curving her back whilst shivering the lowered

wings and puffing her feathers. This solicitation display was never initiated until after the male had approached the female, i.e. the male took the initiative to copulation with the female deciding the outcome. Female copulation solicitations, therefore, always resulted in successful copulations ($n = 110$), except in four cases where the males lost balance. Mounting and cloacal contact lasted no more than two to four seconds. No extra-pair copulations or forced within-pair copulations were observed.

The temporal distributions of male copulation attempts and female-solicited copulations during the nesting cycle are shown in Fig. 1. All male copulation attempts occurred between days -8 and +9, while female-solicited copulations were restricted to a shorter period from day -6 to day +3. The shorter period during which females solicited copulations could reflect their fertile period. The rate of male copulation attempts varied significantly among the three periods of the nesting cycle ($\chi^2 = 8.00$, $df = 2$, $n = 4$ males for each period, $p < 0.02$), and peaked in the pre-IW period 4 days before egg laying with 2.4 ± 0.5 copulation attempts per hour. The frequency of female-solicited copulations also showed a significant difference between the three periods of the nesting cycle ($\chi^2 = 8.00$, $df = 2$, $n = 4$ females for each period, $p < 0.02$), but peaked one day later than the male attempts at day -3 with 1.7 ± 0.3 solicitations per hour.

The opportunity for males to attempt copulation depended on the frequency of female nest visits, since the majority of copulations took place when the female left the nest. The frequency of female nest visits declined significantly from eight days before first egg until incubation was initiated ($r_s = -1.0$, $n = 10$, $p < 0.003$, Fig. 2). The proportion of female nest visits at which males attempted copulation increased significantly from the pre-IW to the end of the IW period ($r_s = 0.86$,

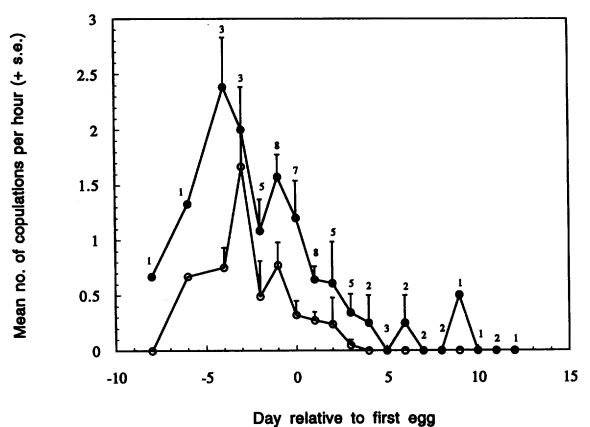


Fig. 1. Mean (+s.e.) copulation frequency relative to the day of first egg (day 0), shown as mean number of male copulation attempts (closed circles) and mean number of female-solicited copulations (open circles). Numbers above the points give the number of focal pairs observed.

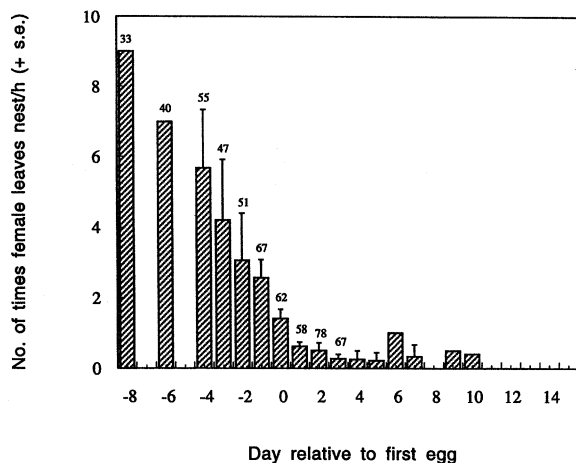


Fig. 2. Number of times the female leaves the nest per hour relative to the day of first egg (day 0). Values are means (+s.e.), sample sizes as shown in Fig. 1. Numbers above columns indicate the percentage of female nest visits utilized by the male to attempt copulation on a given day.

$n = 10$, $p < 0.01$, Fig. 2). However, males did not use all the available opportunities to copulate, as the number of times the female left the nest per hour was always greater than the frequency of male copulation attempts.

When testing the IW hypothesis, which predicts that the chance of fertilizing the next day's egg is greatest shortly after egg laying, it is important to know the time of laying. Females had a mean laying time at 0701 hours \pm 11 min ($n = 12$ females, 17 eggs, range = 0550–0744). Thus, assuming that the time of ovulation in Linnets was comparable to that of domestic birds, the hypothetical IW was predicted to occur within two hours after 0700 hours from day 0 until day +3. There were insufficient data to include the time between 1100 and 1200 hours in the analysis of diurnal variation in copulation behaviour. However, since the IW was expected to occur considerably before 1100 hours it was sufficient to include the observations from 0600 to 1100 hours to test the IW hypothesis. The diurnal variation in male copulation attempts and female solicitations between 0600 and 1100 hours for the pre-IW and the IW period is shown in Fig. 3. In the pre-IW period no significant diurnal variation of copulation behaviour for either males ($\chi^2 = 3.51$, $df = 4$, $n = 6$ males, $p = 0.48$) or females ($\chi^2 = 1.42$, $df = 4$, $n = 6$ females, $p = 0.84$) was found. During the pre-IW period there was no significant diurnal variation in the number of times the female left the nest per hour ($\chi^2 = 1.31$, $df = 4$, $n = 6$ females, $p = 0.86$). Thus, there were equal copulation opportunities throughout the day in this period.

In the IW period males showed a significant peak in copulation attempts between 0800 and 0900 hours ($\chi^2 = 10.88$, $df = 4$, $n = 5$ males, $p = 0.03$, Fig. 3), but there was no significant diurnal variation in female

solicitation frequency in this period ($\chi^2 = 6.13$, $df = 4$, $n = 5$ females, $p = 0.19$, Fig. 3). However, during the IW period females left their nest significantly less frequently between 0600 and 0800 hours ($\chi^2 = 12.3$, $df = 4$, $n = 7$ females, $p < 0.02$) than after 0800 hours, since they spent more time on the nest during egg laying. Thus, there was less opportunity for copulations to occur at this time of day in the IW period. To control for this diurnal variation in copulation opportunity, we weighted the observed copulation frequencies according to the number of times the female left the nest in each hour, i.e. number of copulations per time the female left the nest per hour. When using these weighted data in the analysis of the diurnal variation in copulation behaviour, we obtained the same results as with the original data. The peak in male copulation attempts between 0800 and 0900 hours was still significant ($\chi^2 = 10.3$, $df = 4$, $n = 5$ males, $p < 0.04$), while female solicitation frequency still showed no significant diurnal variation during the IW period ($\chi^2 = 4.5$, $df = 4$, $n = 5$ females, $p = 0.34$). The significantly increased male copulation rate in the second hour after egg laying suggests that males tried to time their copulations to coincide with the IW.

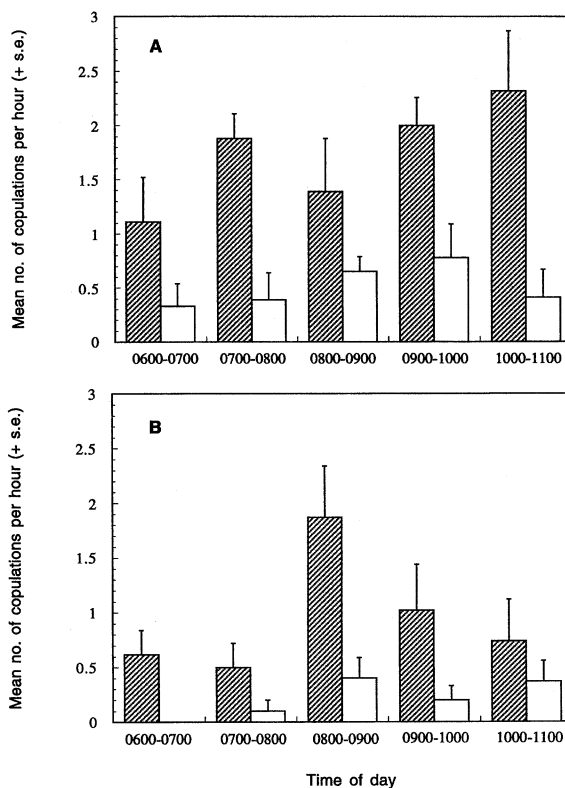


Fig. 3. Diurnal variation in male copulation attempts (hatched bars) and female solicitations (open bars) in (A) the pre-IW ($n = 6$ focal pairs) and (B) the IW period ($n = 5$ focal pairs). The values are means (+s.e.).

Discussion

The data presented here were all obtained by observing the focal pairs near their nest sites. However, these data probably also reflected the copulation behaviour away from the nest site, because copulations were never seen during observations of pairs feeding or collecting nesting material far from the nest site, and all observed copulations were within 40 m of a pair's chosen nest site. Thus, even though focal observations had to be performed near the nest site, they gave a good representation of copulation behaviour in general.

Because information on the duration of sperm storage is not available for the Linnet, the exact start of the fertile period is unknown. The closest related species for which the period of sperm storage is known are the Zebra Finch *Taeniopygia guttata* (10 days; Birkhead et al. 1989) and the Bengalese Finch *Lonchura striata* (8 days; Birkhead 1992). However, the temporal distribution of female solicitations might provide a reasonable estimate of the fertile period, since a female may not be expected to solicit outside her fertile period. Thus, with the mean clutch size of five, the data indicate a fertile period from days -6 to $+3$, which would imply a sperm storage duration of ca 6 days.

The temporal distribution of copulations during the nesting cycle in the Linnet was in line with most other studies of copulation behaviour in birds (see Birkhead and Møller 1992), with most copulations occurring before the laying period. Frequent within-pair copulations, as found in the Linnet, are usually considered as a paternity guard, owing to the advantages of last male sperm precedence and the dilution of possible sperm delivered by competing males (Birkhead and Møller 1992). Even though no extra-pair copulations were observed, the semi-colonial breeding and multiple asynchronous broods in the Linnet could be expected to give ample opportunities for extra-pair copulations (Birkhead and Møller 1992, Møller and Birkhead 1993). In Linnets copulations had to be preceded by female solicitation in order to be successful. Thus, as in most studies of copulation behaviour in birds (e.g. Wagner 1991, Lifjeld and Robertson 1992) the outcome of copulations was fully under the control of females, with only 37% of all male copulation attempts being successful. Hence, the efficiency of frequent copulations as a paternity guard was ultimately under female control. In addition to frequent copulations, male Linnets also used mate guarding by following their female closely as a paternity guard (Drachmann 1997). The efficiency of these two paternity guards in the Linnet is unknown, since currently we have no data on the percentage of extra-pair young in Linnet broods.

The exact time of ovulation is unknown for Linnets, but an IW in the second hour after laying would be comparable to that of domestic birds (Sturkie 1976). Thus, a likely explanation for the observed significant

increase in copulation frequency in the second hour after laying, could be that male Linnets tried to maximize their chances of fertilizing the next day's egg by exploiting the IW. However, an alternative explanation would be that males may be copulating at a high frequency at the first available opportunity every morning. During the IW period this will be immediately after egg-laying, but prior to that it may be shortly after dawn. But ad libitum observations made before 0600 hours in the pre-IW period did not indicate an increased copulation frequency shortly after dawn. Thus, the most likely explanation for the observed increase in male copulation frequency after egg-laying would be that the males tried to exploit the IW. This idea, that birds exploit the IW, has recently been challenged by Birkhead et al. (1996). They argue that the IW is not the optimal time for inseminations in birds, since inseminations undertaken during the IW are relatively unsuccessful compared with those undertaken at other times. This is because the uptake of sperm by the female is reduced immediately before and after oviposition. Thus, according to Birkhead et al. (1996) copulations made during the IW are relatively unlikely to result in fertilization, which should be consistent with field observations showing that very few bird species change their copulatory behaviour during this period. However, the data discussed by Birkhead et al. (1996) are based on artificial insemination experiments, comparing single inseminations by different males. These kinds of data may differ from those obtained from studies of wild birds, where the typical pattern is single copulation versus multiple copulations when comparing extra-pair males with pair males. Even though the IW may not be the optimal time for sperm uptake, inseminations during the IW can still result in fertilization of the next day's egg, as shown by Cheng et al. (1983). Therefore, in populations of wild birds experiencing extra-pair copulation attempts, frequent within-pair copulations during the IW will increase the efficiency of frequent copulations as a paternity guard. Additionally, if inseminations made during the IW result in inefficient use of sperm, male Linnets would not be expected to attempt multiple copulations at all after laying. In the light of this apparent waste of sperm, the males should be receiving some kind of benefit from their increased copulation frequency after laying. Thus, the only explanation we can think of for the observed peak in male copulation attempts in the second hour after laying is the increased chance of fertilizing the next day's egg.

In addition to this study on Linnets only the Mallard, the Barn Swallow *Hirundo rustica*, the Starling *Sturnus vulgaris* and the Aquatic Warbler *Acrocephalus paludicola* have been reported to increase their copulatory behaviour during the time after laying (see Birkhead et al. 1996 for references). The very conspicuous copulation behaviour (in the top of trees) and the semi-colonial breeding in Linnets may be the reason

why male Linnets tried to exploit the IW. Potential extra-pair males were always nearby and they could easily keep track of the fertility status of neighbouring females. However, it is too early to generalize as to why some species but not others try to exploit the IW, since too few detailed studies have been conducted on the IW hypothesis. Future studies on the subject in wild bird populations should try to test the temporal link between copulation behaviour and egg laying for individual birds, not just use mean laying time, since the precise timing of egg laying is important for the testing of this hypothesis.

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