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The insemination window provides a distorted view of sperm competition in birds

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SUMMARY

The aim of this study is to identify and rectify a misunderstanding about the optimal timing of inseminations in birds. In species laying clutches of more than one egg, a copulation during the hour following egg-laying can result in sperm reaching the site of fertilization in time to fertilize the next egg to be laid. Cheng *et al.* (1983) referred to this period as the insemination window and proposed that it was 'an especially favourable period' for males to obtain extra-pair copulations. As stated in their paper, this is true only in terms of the next ovum to be fertilized, but subsequent authors assumed that the insemination window represents a general peak in female fertility and have made predictions about the optimal timing of extra-pair behaviours and paternity guards relative to it. Far from being a general peak in female fertility, we show by a re-analysis of Cheng *et al.*'s data and by using published information on the domestic fowl *Gallus domesticus*, turkey *Gallopavo meleagris* and Muscovy duck *Cairina moschata*, that inseminations either just after egg-laying or just before it are much less likely overall to result in fertilization than inseminations made at other times. The reduced efficacy of inseminations made close to the time of egg-laying occurs because the retention of sperm by females inseminated at this time is low. The fact that inseminations made around the time of egg-laying in the domestic fowl, turkey and Muscovy duck have a reduced probability of fertilization is consistent with the fact that very few wild birds, even those in which sperm competition is intense, alter their copulation or mate guarding behaviour during the insemination window.

1. INTRODUCTION

Sperm competition is widespread in birds and occurs when females copulate with more than one male during a breeding cycle (Birkhead & Møller 1992; Westneat & Webster 1994). The mechanisms of sperm competition, that is, the factors that determine which of two males will fertilize a female's ova have been obscure until recently. Recent studies show that the outcome of sperm competition in birds is determined by the relative number of sperm from different males present at the site of fertilization at the time of fertilization (Birkhead *et al.* 1995*a*; Colegrave *et al.* 1995). This in turn is determined by a number of different factors, including: (i) the relative number of sperm introduced by different males; (ii) the interval between their inseminations; and (iii) the proportion of sperm retained by the female from each ejaculate. The proportion of sperm retained by the female is affected by the timing of insemination relative to oviposition and in a sperm competition situation inseminations made close to oviposition are relatively unsuccessful (Birkhead *et al.* 1995*a*; T. R. Birkhead & J. D. Biggins, in preparation). However, this result appears to contradict the statement by Cheng *et al.* (1983) that the hour following oviposition is an 'especially favourable period' for males to obtain extra-pair copulations.

Cheng *et al.* (1983) referred to the hour following oviposition as the insemination window (IW) because an insemination made during the IW could fertilize the next ovum to be ovulated. The fact that an insemination at this time can result in the fertilization of the next egg to be laid is well established in the domestic fowl *Gallus domesticus* and turkey *Meleagris gallopavo* (Nicolaides 1934; Bobr *et al.* 1964; Howarth 1971; Bakst *et al.* 1994). This effect occurs because during the IW the oviduct is empty and sperm are able to travel up the oviduct, unimpeded by a developing egg, and reach the infundibulum (where fertilization takes place) either before or just after the next ovulation (Bobr *et al.* 1964; Howarth 1971).

Cheng *et al.*'s (1983) study has been influential and subsequent researchers have assumed that the IW is a period of peak fertility for the female, and have used this to generate predictions relating to sperm competition. Specifically, that pair copulations, extra-pair copulations, mate guarding and female solicitation for extra-pair copulations should all be more frequent or intense during the IW (see, for example, Afton 1985; Birkhead *et al.* 1987; Aguilera & Alvarez 1989; Venier & Robertson 1991; Briskie 1992; Chek *et al.* 1993;

Sheldon 1994; Sheldon & Burke 1994; Sorenson 1994). The idea that the IW constitutes a period of peak female fertility has been enhanced by two further sets of observations. First, in many birds copulation is most frequent in the early morning which is also when egg laying tends to occur and so the diurnal pattern of copulation appears to coincide with the IW (Birkhead *et al.* 1987; Birkhead & Møller 1992). Second, females of a number species utter distinctive calls immediately after egg laying, apparently advertising this assumed peak in fertility to promote male–male competition (Thornhill 1988; Montgomerie & Thornhill 1989).

There are three aims of this paper: (i) to show that the IW is not a period of peak fertility in females, by a re-assessment of Cheng *et al.*'s data (§ 2) and an examination of experimental data from other species (§ 3); (ii) to re-evaluate those studies of wild birds that report a change in behaviour during the insemination window; and (iii) to consider the circumstances in which it might pay either sex to focus copulations, or pair males to focus mate guarding, at this time.

2. CHENG *ET AL.*'S EXPERIMENTS RECONSIDERED

The objective of Cheng *et al.*'s (1983) study was to demonstrate that if a female mallard *Anas platyrhynchos* was inseminated within one hour after egg laying, the egg laid the following day could be fertilized by sperm from this insemination. They were not concerned with whether subsequent eggs were fertilized by this insemination (providing there were no subsequent inseminations by another male), as this was already well established. Cheng *et al.*'s (1983) evidence that the IW represents a favourable time for extra-pair copulations was derived from two experiments, which we refer to here as experiment 1 and 2 and consider in turn.

(*i*) **Experiment 1**

Cheng *et al.* showed that when females which did not contain any sperm from previous inseminations were artificially inseminated within 1 h of oviposition, four of 25 (16%) eggs laid the next day were fertile. In contrast, inseminations made more than one hour after oviposition fertilized only 1 of 179 (0.6%) of eggs laid the next day. This experiment demonstrates the existence of an IW in the mallard, as occurs in other species (see above).

The explanation for why inseminations made later than 1 h after laying were less successful than those during the IW is a direct consequence of the processes associated with fertilization and egg formation in the oviduct. The fully formed ovum (the 'yolk') is shed from the ovary into the infundibulum at the top of the oviduct. If sperm are present there, fertilization occurs within 15–20 min of ovulation. After this time the outer perivitelline layer and the first layers of albumen are laid down around the ovum, which sperm are unable to penetrate. The ovum then travels down the oviduct where the membranes and shell are added, and the fully formed egg is laid approximately 24 h after it was ovulated. Within 1 h of laying (oviposition) the next ovum is released from the ovary and the cycle repeats itself (Howarth 1974). Thus if sperm from an IW insemination reach the unfertilized ovum before the outer perivitelline layer and the albumen are laid down, they can result in its fertilization. On the other hand, sperm from an insemination made later than 1 h after ovulation will reach the ovum after the outer perivitelline layer and albumen have been laid down, and therefore cannot fertilize that ovum.

While there is no doubt that inseminations during the hour following oviposition can result in the fertilization of some eggs laid the next day, the general efficacy of IW inseminations has to be assessed by comparing them with inseminations made outside the IW. Although Cheng *et al.* did not do the experiments necessary to examine this (it was not their intention to do so), they do provide some data that allow us to make a comparison. When they made inseminations outside the IW, but still during the laying period, these resulted in the fertilization of 304 of 354 (86%) of eggs laid on the following days, but obviously excluding those laid the next day. As the IW inseminations resulted in only 16% fertilization success (above), this indicates that IW inseminations were relatively unsuccessful. Whereas this suggests that inseminations outside the IW have a higher fertilization success overall, the comparison is not strictly valid. Ideally we should compare the overall proportion of eggs fertilized over exactly the same time period: Cheng *et al.* examined the fertility of eggs only on the day following the IW insemination, and not on subsequent days. While Cheng *et al.*'s data for the mallard cannot be used to show that IW inseminations are less likely to result in fertilization than inseminations made at other times, studies on domestic fowl and turkey do show this effect (see § 3, below)

(*ii*) **Experiment 2**

In this sperm competition experiment Cheng *et al.* (1983: table 4) inseminated females twice with semen from two different genotypes enabling them to assign paternity to the subsequent offspring. Both inseminations were made during the IW using the same volume of semen, 3 d apart. The sperm from the second insemination fertilized five of 11 (45.5%) eggs laid the next day. Although they do not say so explicitly, Cheng *et al.* imply that this result supports their idea that the IW is a favourable time for an extra-pair copulation to fertilize the next egg to be laid.

This experiment demonstrates that an insemination made during the IW can result in fertilization of the next egg even if the female contains sperm from another male. However, this result alone does not show that the IW is an especially favourable time for an extra-pair copulation, for the following reasons. If we assume that the mechanisms of sperm competition in mallards are similar to that proposed for other birds (Birkhead *et al.* 1995*a*; Colegrave *et al.* 1995; see also E. J. A. Cunningham & K. M. Cheng, in preparation), it is clear that far from being an especially favourable time, the

IW is a relatively poor time for insemination. To see why this is the case it is necessary to outline the processes associated with insemination and fertilization and the mechanism of sperm competition. Following insemination a large proportion of sperm are rejected by the female, of those retained, some sperm travel directly to the infundibulum, but most sperm do not and are stored in the sperm storage tubules at the utero-vaginal junction (Howarth 1971; Brillard *et al.* 1987; Brillard & Bakst 1990). Sperm are then released from these tubules at a constant rate over the next few days or weeks (Wishart 1987). If a female is inseminated twice with equal numbers of sperm, then everything else being equal, two factors will affect the ratio of sperm from the two males in the female tract and hence their probability of fertilization. These are: (i) the instantaneous *per capita* rate of sperm loss from the female tract; and (ii) the time interval between the two inseminations: the longer the interval, the more sperm from the first insemination has been lost from the female tract, and the greater the fertilization success of the second male (Lessells & Birkhead 1990; Birkhead *et al.* 1995*a*; Colegrave *et al.* 1995). In Cheng *et al.*'s (1983) experiment, the three day interval between inseminations should have resulted in the second insemination fertilizing more, not fewer eggs than the first. This can be demonstrated quantitatively, thus.

The passive sperm loss model (Birkhead *et al.* 1995*a*) predicts that following the insemination of equal numbers of sperm, both during the IW, but 3 d (72 h) apart, the probability of the second male fathering offspring (p) (on a 'logit' scale) is:

$$p = d + \mu T \qquad (1)$$

Where $d =$ the differential fertilizing capacity between the two genotypes used to assign paternity. $d = -0.096 \pm 0.305$ s.e. (calculated by E. J. A. Cunningham & K. M. Cheng (in preparation) using data from mixed inseminations in Cheng *et al.* (1983)).

$\mu =$ the instantaneous *per capita* rate of loss of sperm from the female tract. The value of $\mu = 0.049$ sperm $h^{-1} \pm 0.0052$ s.e. was derived empirically for the mallard by E. J. A. Cunningham & K. M. Cheng (in preparation) using the methods described by Wishart (1987) and Lessells & Birkhead (1990).

$T =$ the time interval (h) between the two inseminations (72 h).

The predicted value for $p = +3.624 \pm 0.48$ s.e.

The observed proportion of offspring fathered by the second insemination in Cheng *et al.*'s (1983) study is: $(\log_N (5/6)) = -0.167 \pm 0.579$ s.e., which is significantly less than that predicted by the passive loss of sperm ($z = 4.57$, $p < 0.001$). The difference between the observed and predicted results presumably arises because only a small proportion of the sperm from the second insemination reach the infundibulum in sufficient time to fertilize the recently ovulated ovum.

3. THE INSEMINATION WINDOW IN OTHER SPECIES

There is abundant evidence from studies of poultry (domestic fowl, turkey and Muscovy duck *Cairina moschata*) that inseminations made near the time of egg laying are relatively unsuccessful (references in Brillard *et al.* 1987; Raud & Faure 1990). For example, in domestic fowl inseminations made away from the time of laying resulted in 90 % fertility, compared with 50 % for those made 1 h after laying (Brillard *et al.* 1987). In turkeys the equivalent values were 96 and 66 %, respectively (Christensen & Johnson 1977). More importantly in the present context, is the reduction in fertilization success recorded in two sperm competition experiments in the domestic fowl in which one of two inseminations occurred within 1 h of egg-laying (Leman 1975; Compton *et al.* 1978). We estimated the reduction in fertilization success for IW inseminations (using formulae 2 and 3 in Birkhead *et al.* 1995): for both studies inseminations made during the IW were 66–70 % less successful than inseminations made at other times. Johnston *et al.* (1984) showed the same effect in both the domestic fowl and turkey.

The actual mechanism responsible for reduced fertilization success of inseminations made soon after laying has been determined experimentally and comprises a reduction in the proportion of sperm retained in the cloaca or vagina as a consequence of the contractions associated with oviposition (Brillard *et al.* 1987; see also Verma & Cherms 1967; Brillard & Bakst 1990). As mentioned above, inseminations made an hour or so before laying are also less likely to result in fertilization. The mechanism responsible for reduced sperm uptake by the female here is the presence of the hard-shelled egg in the shell gland (see, for example, Christensen & Johnston 1977). The precise durations of the time either side of oviposition in which the uptake of sperm and hence the probability of fertilization reduced, is not known for any species, but it appears to be at least 2 h, and it may not be symmetrical (see Leman 1975; Brillard *et al.* 1987; Raud & Faure 1990).

4. COPULATION, MATE GUARDING AND THE INSEMINATION WINDOW

Despite the apparent coincidence between the diurnal timing of oviposition and copulations in birds (Birkhead *et al.* 1987), in many species the early morning peak in copulation frequency occurs well before ovulation starts. Moreover, in many species copulations usually decrease in frequency or cease altogether once egg laying has started so that the IW is rarely used (reviewed in Birkhead & Møller 1993; see also Venier & Robertson 1991; Westneat 1993; Sheldon & Burke 1994). In a number of studies authors have specifically looked for changes in behaviour associated with the IW and failed to find any. Even in polygynandrous species in which intense sperm competition exists and copulations often continue through the egg laying period (Birkhead & Møller 1992), there is no evidence that copulations occur more often during the insemination window (e.g. Smith's Longspur *Calcarius pictus*, Briskie 1992; Dunnock *Prunella modularis*, Alpine Accentor *P. collaris*, Davies *et al.* 1996).

However, there are a few studies (in addition to Cheng *et al.* 1983) in which observers have reported an increase in either copulatory or mate guarding behaviour during the time after laying.

1. Barn swallow *Hirundo rustica*: copulations and copulation attempts were more common following egg laying (Møller 1987).
2. European starling *Sturnus vulgaris*: male extra-pair activity tended to be more frequent and mate-guarding more intense in the late morning, following egg laying (R. Pinxten & M. Eens, unpublished data).
3. Chaffinch *Fringilla coelebs*: Sheldon (1994) recorded an increase in mate guarding intensity during the hour or so after females had laid, but there was no increase in copulations or in soliciting by females during this time (Sheldon & Burke 1994).
4. Aquatic warbler *Acrocephalus paludicola*: sperm competition in this species is intense, many females having each of their five or six offspring fathered by a different male (Schulze-Hagen *et al.* 1993). Copulations are virtually impossible to observe in the wild, and observations of two males in captivity showed that copulations comprised: (i) multiple inseminations during unusually protracted mounting (in this respect it is probably no coincidence that males have relatively enormous testes and seminal glomera (Schulze-Hagen *et al.* 1995)); and (ii) before laying started copulations occurred most often in the evening, but once egg laying began they were more common immediately after the female had laid in the morning, suggesting that the birds used the insemination window to fertilize the next day's egg (Schulze-Hagen *et al.* 1995). However, direct evidence for this (which would be difficult to obtain) is lacking.

We suggest that all these cases of an apparent link between copulation or mate guarding and egg-laying requires closer scrutiny. In none of the studies was the temporal link between the behaviour and egg-laying of individual birds made explicit. This is because in all studies, except the aquatic warbler in captivity, the precise timing of egg-laying was not known. Moreover, in the swallow study it is not clear whether the data on copulation behaviour are derived from the laying period, the pre-laying period or both. Even if the association between behaviour and egg-laying in all four of these studies is statistically robust, each case represents only one out of four possible effects (see § 1) that could possibly occur. Studies claiming an association between the IW and behaviour need to present their data carefully, and to consider the functional significance of such an effect in the light of the information presented here.

4. COSTS AND BENEFITS OF IW INSEMINATIONS

As IW inseminations result in many fewer sperm being taken up by the female and a corresponding reduction in the likelihood of fertilization compared with inseminations at other times, it will rarely be worthwhile for pair members to copulate during this period. Indeed, if they can copulate at other times, the IW would be best avoided. Even for extra-pair copulations IW inseminations would only be worthwhile under certain conditions. Individuals involved in extra-pair copulations face a trade-off between: (i) copulating during IW when they have a probability (albeit quite low) of fertilizing the next day's egg (and subsequent eggs); and (ii) copulating outside the IW when they have a much greater probability of fertilizing all eggs, except the next one to be laid.

The circumstances under which the pay-off from an IW insemination might exceed that from an insemination at another time are limited but include the following:

1. If the IW is the only time an extra-pair male and female can engage in copulation, because of some behaviour by the female or the male partner. This is precisely what Cheng *et al.* (1983) suggested for the mallard. In this species extra-pair males intercepted and attempted extra-pair copulations with females as they returned, unaccompanied by their partner, to the water after egg laying.
2. If the female is about to ovulate the final egg of the clutch, and the IW is the last opportunity for an extra-pair fertilization. Clutch size varies from 1–17 eggs in different bird species (Lack 1968). For those laying small clutches the opportunities for individuals to engage in extra-pair copulations during the egg-laying period will obviously be limited. A prediction arising from this is that IW-extra-pair copulations will be relatively more frequent for those species laying small clutches. The likelihood of success however will still depend on the number the partner's sperm present in the female's tract. For species with a low within-pair copulation rate, a high rate of sperm loss and long interval since the last pair copulation, an IW insemination will have a relatively high chance of fertilizing the next ovum.
3. If a male could inseminate sufficient sperm to offset the reduced uptake during the IW, as may occur in the aquatic warbler (see above). Although in some species extra-pair copulations result in the insemination of relatively large numbers of sperm (Birkhead *et al.* 1995*b*), everything else being equal, an IW insemination effectively wastes sperm as fertilization success would be greater if the insemination occurred outside the IW.

In conclusion, Cheng *et al.*'s experiments confirm an insemination window exists in the mallard, just as in the domestic fowl and turkey, and presumably most other birds. Cheng *et al.*'s experiments also show that an IW insemination can result in mixed paternity in a sperm competition situation. Subsequent authors appear to have misinterpreted these results: Cheng *et al.* proposed that the IW represents a peak in fertility in terms only of the next egg to be laid, but other workers have assumed that the IW represents a general peak in female fertility. In fact, as studies of the domestic fowl, turkey and Muscovy duck have clearly demonstrated, IW inseminations are relatively unsuccessful compared with those made at other times. This is because the uptake of sperm by the female is reduced immediately before and after oviposition. The fact that IW inseminations result in the reduced uptake of sperm and are therefore relatively unlikely to result in fertilization is consistent with field observations showing that very few bird species change their copulatory behaviour during this period.

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REFERENCES

Afton, A. 1985 Forced copulation as a reproductive strategy of male lesser scaup: a field test of some predictions. *Behaviour* **92**, 146–167.

Aguilera, E. & Alvarez, F. 1989 Copulations and mate guarding of the spoonbill (*Platalea leucorodia*). *Behaviour* 110, 1–22.

Bakst, M. R., Wishart, G. J. & Brillard, J. P. 1994 Oviducal sperm selection, transport, and storage in poultry. *Poultry Sci. Rev.* 5, 117–143.

Birkhead, T. R., Atkin, L. & Møller, A. P. 1987 Copulation behaviour of birds. *Behaviour* 101, 101–138.

Birkhead, T. R., Fletcher, F., Pellatt, E. J. & Staples, A. 1995*b* Ejaculate quality and the success of extra-pair copulations in the zebra finch. *Nature, Lond.* **377**, 422–423.

Birkhead, T. R. & Møller, A. P. 1992 *Sperm competition in birds: evolutionary causes and consequences*. London: Academic Press.

Birkhead, T. R. & Møller, A. P. 1993 Why do male birds stop copulating while their partners are still fertile? *Anim. Behav.* **45**, 105–118.

Birkhead, T. R., Wishart, G. J. & Biggins, J. D. 1995*a* Sperm precedence in the domestic fowl. *Proc. R. Soc. Lond.* B **261**, 285–292.

Bobr, L. W., Lorenz, F. W. & Ogasawara, F. X. 1964 Distribution of spermatozoa in the oviduct and fertility in domestic birds. 1. Residence sites of spermatozoa in fowl oviducts. *J. Reprod. Fert.* **8**, 39–47.

Brillard, J. P. & Bakst, M. R. 1990 Quantification of spermatozoa in the sperm-storage tubules of turkey hens and its relation to sperm numbers in the perivitelline layer of eggs. *Biol. Reprod.* **43**, 271–275.

Brillard, J. P., Galut, O. & Nys, Y. 1987 Possible causes of subfertility in hens following insemination near the time of ovulation. *Brit. Poult. Sci.* **28**, 307–318.

Briskie, J. V. 1992 Copulation patterns and sperm competition in the polygynandrous Smith's longspur. *Auk* **109**, 563–575.

Chek, A. A., Lifjeld, J. T. & Robertson, R. J. 1993 Captive study of copulation in Pied Flycatcher *Ficedula hypoleuca*. *Fauna norv. Ser. C, Cinclus* **16**, 67–73.

Cheng, K. M., Burns, J. T. & McKinney, F. 1983 Forced copulation in captive mallards. III. Sperm competition. *Auk* **100**, 302–310.

Christensen, V. L. & Johnston, N. P. 1977 Effect of time of day of insemination and the position of the egg in the oviduct on the fertility of turkeys. *Poultry Sci.* **56**, 458–462.

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.

Compton, M. M., Van Krey, H. P. & Siegel, P. B. 1978 The filling and emptying of the uterovaginal sperm-host glands in the domestic hen. *Poultry Sci.* **57**, 1696–1700.

Davies, N. B., Hartley, I. R., Hatchwell, B. J. & Langmore, N. E. 1996 Female control of copulations to maximize male help: a comparison of polygynandrous alpine accentors, *Prunella collaris*, and dunnocks *P. modularis*. *Anim. Behav.* **51**, 27–47.

Howarth, B. 1971 Transport of spermatozoa in the reproductive tract of turkey hens. *Poultry Sci.* **50**, 84–89.

Howarth, B. 1974 Sperm storage as a function of the female reproductive tract. In *The oviduct and its functions* (ed. A. D. Johnson & C. E. Foley). New York: Academic Press.

Johnston, N. P., Warner, D., Buckener, R. & Arscott, G. 1984 Effect of oviducal egg position at insemination on selective fertilization of poultry. *Proc. 17th World's Congress*. 198–199.

Lack, D. 1968 *Ecological adaptations for breeding in birds*. London: Chapman and Hall.

Leman, A. D. 1975 *A study of the factors that may affect heterospermic dominance*. Unpubl. PhD thesis, University of Illinois at Urbana-Champaign. p84.

Lessells, C. M. & Birkhead, T. R. 1990 Mechanisms of sperm competition in birds: mathematical models. *Behav. Ecol. Sociobiol.* **27**, 325–337.

Møller, A. P. 1987 Behavioural aspects of sperm competition in swallows *Hirundo rustica*. *Behaviour* **100**, 92–104.

Montgomerie, R. & Thornhill, R. 1989 Fertility advertisement in birds: a means of inciting male-male competition? *Ethology* **81**, 209–220.

Nicolaides, C. 1934 Fertility studies in poultry. *Poult. Sci.* **13**, 179–183.

Raud, H. & Faure, J. M. 1990 Rhythmic occurrence of sexual behaviour and egg laying activity of Muscovy ducks. *Brit. Poultry Sci.* 31, 23–32.

Schulze-Hagen, K., Swatschek, I., Dyrcz, A. & Wink, M. 1993 Multiple vaterschaften in bruten des seggenrohrsangers *Acrocephalus paludicola*: erste ergebnisse des DNA-fingerprintings. *J. Orn.* **134**, 145–154.

Schulze-Hagen, K., Leisler, B., Birkhead, T. R. & Dyrcz, A. 1995 Prolonged copulation, sperm reserves and sperm competition in the aquatic warbler *Acrocephalus paludicola*. *Ibis* **137**, 85–91.

Sheldon, B. C. 1994 Timing and use of paternity guards by male chaffinches. *Behaviour* **129**, 79–97.

Sheldon, B. C. & Burke, T. 1994 Copulation behaviour and paternity in the chaffinch. *Behav. Ecol. Sociobiol.* **34**, 149–156.

Sorensen, L. G. 1994 Forced extra-pair copulation and mate guarding in the white-cheeked pintail: timing and trade-offs in an asynchronous breeding duck. *Anim. Behav.* **48**, 535–549.

Thornhill, R. 1988 The jungle fowl hen's cackle incites male competition. *Verh. Dtsch. Zool. Ges.* **81**, 145–154.

Venier, L. A. & Robertson, R. J. 1991 Copulation behaviour of the tree swallow, *Tachycineta bicolor*: paternity assurance in the presence of sperm competition. *Anim. Behav.* **42**, 939–948.

Verma, O. P. & Cherms, F. L. 1965 The appearance of sperm and their persistency in storage tubules of turkey hens after a single insemination. *Poultry Sci.* **44**, 609–613.

Westneat, D. F. 1993 Temporal patterns of within-pair copulations, male mate-guarding, and extra-pair events in eastern red-winged blackbirds (*Agelaius phoeniceus*). *Behaviour* **124**, 267–290.

Westneat, D. F. & Webster, M. S. 1994 Molecular analysis of kinship in birds: Interesting questions and useful techniques. In *Molecular ecology and evolution: approaches and applications* (ed. B. Schierwater, B. Streit, G. P. Wagner & R. DeSalle). Basel: Birkhauser Verlag.

Wishart, G. J. 1987 Regulation of the length of the fertile period in the domestic fowl by numbers of oviductal spermatozoa as reflected by those trapped in laid eggs. *J. Reprod. Fert.* **80**, 493–498.

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