Pre- and post-insemination episodes of sexual selection in the fowl, *Gallus g. domesticus*

T Pizzari¹, DP Froman² and TR Birkhead³

¹Section of Ethology, Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Skara, SE-532-23, Sweden; ²Department of Agriculture – Poultry Science, University of Georgia, Athens, GA 30602-2772, USA; ³Department of Animal and Plant Sciences, University of Sheffield, S10 2TN, UK

Although much attention has been recently directed to sexual selection arising after insemination from sexual promiscuity, little is known about the mechanisms determining reproductive success after insemination, and the way these mechanisms interact with each other and with selective mechanisms occurring before insemination: mate choice and mate acquisition. Here, we briefly review the findings of an on-going study investigating the mechanisms generating variation in reproductive success at both a pre- and a post-insemination stage in the domestic fowl. Female preference consistently favours socially dominant males before and

after insemination. However, although social status mediates the number of sperm that a male inseminates into a female, dominant males may inseminate sperm of lower fertilising quality than their subordinates. We argue that mitochondrial genes may contribute to determine sperm quality, and speculate that the maternal control of mitochondrial genes may prevent sexual selection from operating on males, thus explaining both the lack of a positive correlation between social dominance and sperm quality and the maintenance of variation in male quality in the fowl.

Heredity (2002) **88**, 112–116. DOI: 10.1038/sj/hdy/6800014

Keywords: sperm competition; female cryptic choice; fertilising efficiency; fowl

Introduction

Sexual selection acts on heritable variation in reproductive success. Anisogamy and the consequential discrepancy between the reproductive rates of males and females (Clutton-Brock and Parker, 1992) generates different reproductive interests in the two sexes. Males generally increase reproductive success by out-competing rivals over fertilisation opportunities (Darwin, 1871; Andersson, 1994). The reproductive success of females, on the other hand, is often limited by the number of eggs they can produce (Bateman, 1948; Trivers, 1972). However, an important source of variation in female reproductive success may be determined by male genotype and/or phenotype, through the effect of the paternal genes expressed in the offspring (Pomiankowski *et al*, 1991; Andersson, 1994; Chippindale *et al*, 2001), the direct effect of the paternal phenotype on the mother and her offspring (Andersson, 1994; Qvarnstrom *et al*, 2000), and through differential maternal investment in relation to the paternal phenotype (Sheldon, 2000).

Until relatively recently most organisms were believed to be sexually monogamous and sexual selection was expected to operate mainly through episodes leading to the insemination of an ejaculate into a female, namely mate choice and mate acquisition. However, molecular evidence combined with behavioural studies has indicated that sexual promiscuity is typical among most plant

and animal species (Birkhead and Møller, 1998). Sexual promiscuity creates potential for sexual selection to continue after insemination through: (i) the competition between the ejaculates of different males for the fertilisation of a set of eggs, sperm competition (Parker, 1970), and (ii) the selection by the female or her ova of the sperm of a particular male for fertilisation, female cryptic choice (Thornhill, 1983; Eberhard, 1996). The realisation that widespread sexual promiscuity extends sexual selection beyond insemination has important implications for the understanding of the evolution of reproductive fitness in males and females. The reproductive fitness of a male is determined not only by traits that influence the number of females inseminated but also by traits which influence the ability of a male's ejaculates to fertilise eggs in a competitive situation (Parker, 1970; Pizzari and Birkhead, 2001). Similarly, when sperm competition occurs, female traits are expected to evolve which allow females to preferentially utilise the sperm of the preferred males for fertilisation, particularly in species where males can coerce females into copulating, thus limiting females' opportunities to select copulation partners (Eberhard, 1996).

Reproductive fitness is therefore determined by the complex interactions of traits selected in males and females during different episodes of selection before and after insemination. The way in which different sexually selected traits affect the fitness of males and females and the way these traits interact at both a phenotypic and a genetic level has profound implications for the evolution of the reproductive fitness and the evolutionary trajectories of both sexes, and is the focus of much current work in sexual selection.

The aim of this study is to illustrate the relationship between male and female reproductive strategies adopted before and after insemination in a typically sexually promiscuous bird, the feral fowl, *Gallus g. domesticus*.

The study subject

The red junglefowl, *Gallus gallus*, and feral populations of its domesticated descendant, the domestic fowl, live in multi-male multi-female, socially structured groups which are characterised by high levels of sexual promiscuity despite the efforts of dominant males to monopolise access to females (McBride *et al*, 1969; Cheng and Burns, 1988; Thornhill, 1988; Collias and Collias, 1996).

The study was conducted on a free-ranging unconstrained population of feral fowl at the research station of Stockholm University, Sweden, between 1998 and 2000. The study population belongs to a breed of domestic fowl from south-east Asia which has been subjected to typically relaxed artificial selective pressures and is morphologically and behaviourally very similar to the red junglefowl (Pizzari and Birkhead, 2001; Schütz and Jensen, 2001). We studied the sexual behaviour of individually marked birds in groups comprising of 10–13 males and 21–28 females. In addition, we investigated the effect of sperm quality on male reproductive success using a random-bred population of New Hampshire fowl (base population $n = 242$) housed at Oregon State University (Froman *et al*, 1999).

Pre-insemination strategies

Males

Socially dominant males experienced higher copulation success and privileged access to copulation opportunities than their subordinates (Pizzari and Birkhead, 2000; Pizzari, 2001; Pizzari, MS). This advantage was partly explained by differential ability to attract and seduce females and by the ability of dominant males to disrupt copulations initiated by their subordinates (Pizzari, 2001). Male fowl provide females with food as part of their courtship display (courtship-feeding). In the study population dominant males performed more courtship-feeding than subordinate males (Pizzari, MS). In addition, when males were experimentally provided with food, the probability that they courtship-fed females was positively correlated with male social status (Pizzari, MS). Male fowl are also more vigilant than females and signal potential predators with specific alarm calls. Females may therefore benefit by foraging in proximity of a vigilant male. Dominant males invested significantly more time in vigilance than subordinate males (Pizzari, MS). Dominant males, but not subdominants, also produced alarm calls at a rate which was positively correlated with the amount of time spent vigilant, suggesting that alarm calls by dominant males reliably indicate their vigilance effort, and that the alarm calls of dominant males, unlike the calls of subordinate males, provide females with reliable cues on the presence of potential predators (Pizzari, MS). This result is consistent with accounts of females preferentially associating with dominant males in populations of feral and red junglefowl (McBride *et al*, 1969; Thornhill, 1988). Despite the competitive advantage

of dominant males, the effect of social dominance on copulation success is often weak and most males enjoy some copulation success (Pizzari, 1999, 2001; Pizzari *et al*, MS; T Pizzari, TR Birkhead, unpublished data).

Females

Female fowl showed a marked preference for socially dominant copulation partners. In the study population female copulatory behaviour biased copulation success in favour of dominant males. The probability of females soliciting copulation was positively correlated with male social status (Pizzari, 2001). In addition, females were also more likely to resist the sexual advances of subordinate males (Pizzari, 2001). The direct selection of socially dominant males through differential solicitation and resistance had a strong influence on copulation success (Pizzari, 2001). However, the fact that males are larger and consistently socially dominant over females (Pizzari, 1999) allows males to coerce females into copulating, thus limiting female opportunities to directly select copulation partners. When there are limited opportunities to directly select copulation partners, females may bias copulation success indirectly through the manipulation of male sexual behaviour (Wiley and Poston, 1996). For example, females may promote competition for fertilisation among males, which in turn may increase the success of dominant males. Females may elicit competition by signalling the time when inseminations are most likely to result in fertilisation or by signalling to other males that a copulation is taking place. Female fowl produce two calls which could bias copulation success in favour of dominant males: the post-oviposition cackle, and the distress call. The post-oviposition cackle is a loud repetitive call uttered by a female following egg-laying. Based on the assumption that the hour immediately following egg-laying is particularly favourable for an insemination to result in the fertilisation of the successive eggs, it has been suggested that this vocalisation may signal a peak in fertility and thus trigger male sexual behaviour and competition for the insemination of a cackling female (Thornhill, 1988).

The distress call is often associated with copulation (Collias, 1987; Thornhill, 1988). Due to its tight association with copulation (Thornhill, 1988), the distress call may influence male sexual behaviour and thus allow for indirect partner selection.

We tested the effect of both the post-oviposition cackle and the distress call on male behaviour. We found no evidence that post-oviposition cackling results in the indirect selection of socially competitive copulation partners. In fact, this vocalisation was associated with a significantly lower probability of a female obtaining a copulation in our study. These results are consistent with several artificial insemination studies showing that the time immediately following oviposition is in fact an unfavourable time for an insemination to fertilise eggs (Birkhead *et al*, 1996) and suggest that cackling may discourage males from attempting to copulate (Pizzari and Birkhead, 2001). The distress call, on the other hand, had a strong influence on the outcome of a copulation. Females were more likely to utter the call when approached by low-ranking males attempting to copulate, and the distress call attracted males, thus increasing the probability of other males detecting a copulation. In addition, males dominant over a copulating male were

likely to disrupt the copulation and to inseminate the calling female. Therefore, differential distress calling allowed females to further bias copulation success in favour of dominant males (Pizzari, 2001).

Post insemination strategies

Males

Despite the fact that copulation success is skewed in favour of dominant males, the effect of social dominance is limited and most subdominant males secure some copulations. Consequently, the risk of sperm competition is typically high and sperm competition can be very intense in feral fowl populations (Jones and Mench, 1991; Pizzari *et al*, MS). When sperm competition occurs, the fertilising efficiency of ejaculates contributes to influence male reproductive success (Parker, 1998; Pizzari and Birkhead, 2001). In general, two factors determine the fertilising efficiency of an ejaculate: (i) the number and (ii) the quality of sperm inseminated.

Number of sperm inseminated: Successive inseminations deplete the sperm reserves of a male (Birkhead *et al*, 1988; Birkhead, 1991). In systems where males need to inseminate a relatively high number of sperm to out-compete the ejaculates of their rivals and where there are multiple copulation opportunities, sperm depletion may limit male reproductive success (Dewsbury, 1982). Most male fowl face intense sperm competition and have the opportunity to inseminate multiple females, strongly suggesting that sperm depletion under sperm competition exerts an intense selective pressure on this species. This is consistent with the fact that male red junglefowl have the largest relative testes mass among galliformes (Pizzari, 1999), indicating an evolutionary response to sperm depletion and sperm competition. When the sperm investment in the current insemination constrains a male's investment in future inseminations, males are expected to allocate sperm differentially according to the reproductive value of an insemination and strategic sperm allocation may evolve (Dewsbury, 1982; Parker, 1998). An obvious response to sperm depletion would be to limit the sperm investment in a particular female in order to be able to inseminate enough sperm into other females. We tested this idea by providing individual male fowl with the opportunity to copulate *ad libitum* with a female. To test whether males limit their sperm investment in the current copulation partner to save sperm for future females we replaced the sexually familiar female with a new one after the male ceased inseminating the familiar female. If males are genuinely sperm depleted they are not expected to be capable of inseminating new females, but if they allocate sperm strategically we expect sperm investment to be renewed with a new female. Over successive copulations, male fowl progressively reduced their sperm investment in a female and, when a new female was presented increased, the number of sperm inseminated, indicating that male fowl respond to the risk of sperm depletion under sperm competition through strategic sperm allocation to different females (Pizzari *et al*, MS). Another way through which males are predicted to maximise reproductive success under sperm competition is to invest sperm differentially according to the probability that an ejaculate compete with the ejacu-

late of another males (risk of sperm competition), and according to the number of males that inseminate the same female, and whose ejaculates compete for the fertilisation of the same set of eggs (intensity of sperm competition, Parker, 1998). We are currently testing these predictions.

Quality of sperm inseminated: Another trait, in addition to sperm numbers, that plays an important role in determining fertilisation under sperm competition, is the quality of sperm inseminated (Pizzari and Birkhead, 2001). Sperm quality measured as 'sperm mobility', an *in vitro* assay which measures the ability of sperm to penetrate a solution of an inert medium (Froman *et al*, 1999; Froman and Feltmann, 2000). Sperm mobility is a normally distributed trait which is stable and significantly repeatable within males (Froman *et al*, 1999; Froman and Feltmann, 2000). An artificial insemination experiment where the same number of sperm from a male with average-, and from a male with high-sperm mobility were mixed and artificially inseminated into the same female revealed that, when competing with low mobility ejaculates of the same size, high mobility ejaculates fertilised a disproportionate number of eggs (Birkhead *et al*, 1999).

In addition, we investigated the mechanisms through which sperm number and sperm mobility convey a competitive advantage to an ejaculate. A critical factor in fertilisation success in birds is the ability of sperm to ascend the female vagina and enter the utero-vaginal sperm storage tubules (Wishart, 1987). Therefore, we investigated the effect of both sperm mobility and the number of sperm inseminated on sperm storage and the rate at which sperm are lost from the female sperm storage tubules. Within 15 minutes following fertilisation, the ovum is covered with a proteinic layer, the outer perivitelline layer, which traps any sperm present in the infundibulum and around the ovum at the time of fertilisation (Bakst, 1993). Trapped spermatozoa hydrolyse the perivitelline layer and thus the number of perforations present on the perivitelline layer of eggs laid over successive days following an insemination provides an accurate measure of how many sperm of the inseminated ejaculate initially reach the sperm storage tubules and the rate at which they are released from the tubules over successive days (Wishart, 1987; Brillard, 1993). We partitioned variance in the number of sperm reaching the sperm storage tubules and in the rate of sperm loss between the mobility and the number of sperm inseminated by artificially inseminating 100 million sperm from 10 different males which differed in the mobility of their ejaculates into 10 females per male (100 females in total), and then by replicating the same experiment inseminating 25 million of sperm from the same males. We found that the number of sperm inseminated positively affected the number of sperm initially reaching the sperm storage tubules of a female (as expected, see Brillard, 1993), and that crucially, sperm mobility positively affected the rate of sperm loss from the sperm storage tubules, with sperm from high mobility ejaculates being lost more slowly and hence maintaining their fertilising power over a longer period of time (Froman *et al*, MS).

Although most males have the opportunity to copulate with multiple females, access to copulations is typically mediated by male social dominance, dominant males enjoying privileged access to females and disrupting

copulations initiated by the subordinates. The number of sperm inseminated by a male into a female is therefore likely to be mediated by his social status. We tested the phenotypic relationship between the two factors that contributed to determine fertilising efficiency in male fowl: male social status and sperm mobility. We did this by allowing pairs of males which produced ejaculates of either high or average mobility to establish a dominance relationship and found that the males that produced ejaculates of lower mobility were significantly more likely to become the dominant member of these pairs (Froman *et al*, MS).

Females

Consistent with anecdotal information (R Thornhill, cited in Birkhead and Møller, 1992), we found that female feral fowl are able to eject semen through cloacal contractions immediately following an insemination. Sperm ejection can be one of the mechanisms through which females may bias paternity after insemination (Eberhard, 1996). Because female fowl preferred to copulate with high-ranking males we tested whether the probability of sperm ejection was equal for the ejaculates of dominant and subordinate males. The probability of sperm ejection was significantly and negatively correlated with the status of a male: more dominant males experienced a decreased probability of having their ejaculates ejected by females (Pizzari and Birkhead, 2000). To determine the causality of this relationship we removed some males, including the top-, and the bottom-ranking males, to experimentally manipulate the social status of the remaining males. Consistent with the idea that female fowl eject the sperm of subordinate males, we found that those males that occupied a higher rank after the manipulation experienced a decreased probability of sperm ejection (Pizzari and Birkhead, 2000). These results suggest that female fowl are able to re-iterate their choice of partners after insemination.

Evolutionary implications

Three distinct sexually selective episodes consistently favour male social dominance in the domestic fowl: (i) pre-insemination male-male competition, (ii) pre-insemination female choice, and (iii) post-insemination female choice. However, in another episode of sexual selection, male-male competition after insemination (ie sperm competition), male social dominance was not phenotypically linked (at least not positively) to sperm mobility, the other male trait determining fertilising efficiency. Competitive ability underlying male social dominance shows significant additive variation in the fowl (Craig *et al*, 1965). Similarly, we found that sperm mobility is highly heritable (Froman *et al*, MS). The additive genetic variation observed in social dominance and sperm mobility, combined with highly interdependent function of these traits, is expected to create potential for positive correlational post-insemination sexual selection (Lande and Arnold, 1983). The mechanisms that prevent social dominance and sperm mobility from working in unison are unknown. However, sperm mobility may be largely maternally transmitted, since a heritability analysis revealed a strong maternal genetic effect on sperm mobility genetically independent from the autosomal effect (Froman *et al*, MS), suggesting that mitochondrial

genes may contribute to determine sperm mobility (Froman *et al*, MS). This finding would be consistent with recent studies of human sperm (Ruiz-Pesini *et al*, 2000) and indicates the possibility that sexual selection may be prevented from acting on sperm mobility along the male line, thus contributing to maintain variability in male quality. In the future it will be crucial to test the extent to which mitochondrial genes control sperm mobility in the fowl.

References

- Andersson M (1994). *Sexual Selection*. Princeton University Press: Princeton.
- Bakst MR (1993). Oviducal sperm storage in poultry. A review. *Repro Fertil Devel* 5: 595–599.
- Bateman AJ (1948). Intra-sexual selection in *Drosophila*. *Heredity* 2: 349–368.
- Birkhead TR (1991). Sperm depletion in the Bengalese finch, *Lonchura striata*. *Behav Ecol* 2: 267–275.
- Birkhead TR, Møller AP (1992). *Sperm Competition in Birds. Evolutionary Causes and Consequences*. Academic Press: London.
- Birkhead TR, Møller AP (eds) (1998). *Sperm Competition and Sexual Selection*. Academic Press: London.
- Birkhead TR, Pellatt JE, Hunter FM (1988). Extra-pair copulation and sperm competition in the zebra finch. *Nature* 334: 60–62.
- Birkhead TR, Cunningham EJA, Cheng KM (1996). The insemination window provides a distorted view of sperm competition in birds. *Proc Royal Soc Lond Series B* 263: 1187–1192.
- Birkhead TR, Martínéz JG, Burke T, Froman DP (1999). Sperm mobility predicts outcome of sperm competition in the domestic fowl. *Proc Royal Soc Lond Series B* 266: 1759–1764.
- Brillard JP (1993). Sperm storage and transport following natural mating and artificial insemination. *Poultry Sci* 72: 923–928.
- Cheng KM, Burns JT (1988). Dominance relationship and mating behavior of domestic cocks: a model to study mate-guarding and sperm competition in birds. *Condor* 90: 697–704.
- Chippindale AK, Gibson JR, Rice WR (2001). Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. *Proc Nat Acad Sci USA* 98: 1671–1675.
- Clutton-Brock TH, Parker GA (1992). Potential reproductive rates and the operation of sexual selection. *Quart Rev Biol* 67: 437–456.
- Collias NE (1987). The vocal repertoire of the red jungle fowl: a spectrographic classification and the code of communication. *Condor* 89: 510–524.
- Collias NE, Collias E (1996). Social organization of a red jungle-fowl, *Gallus gallus*, population related to evolutionary theory. *Animal Behav* 51: 1337–1354.
- Craig JV, Orhan LL, Guhl AM (1965). Genetic selection for social dominance ability in chicks. *Animal Behav* 13: 114–131.
- Darwin C (1871). *The Descent of Man, and Selection in Relation to Sex*. John Murray: London.
- Dewsbury DA (1982). Ejaculate cost and male choice. *Am Naturalist* 119: 601–610.
- Eberhard WG (1996). *Female Control: Sexual Selection by Female Cryptic Choice*. Princeton University Press: Princeton.
- Froman DP, Feltmann AJ (2000). Sperm mobility: phenotype in roosters (*Gallus domesticus*) determined by concentration of motile sperm and straight line velocity. *Biol Reprod* 62: 303–309.
- Froman DP, Feltmann AJ, Rhoads ML, Kirby JD (1999). Sperm mobility: a primary determinant of fertility in the fowl (*Gallus domesticus*). *Biol Reprod* 61: 400–405.
- Froman DP, Pizzari T, Feltmann AJ, Castillo-Suarez H, Birkhead TR (MS). Phenotypic dissection of male fertilising efficiency in a bird. *Proc Royal Soc Lond Series B* (Submitted).
- Jones MEJ, Mench JA (1991). Behavioral correlates of male mating success in multisire flock as determined by DNA fingerprinting. *Poultry Sci* 70: 1493–1498.

- Lande R, Arnold SJ (1983). The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.
- McBride G, Parer IP, Foenander F (1969). The social organisation and behaviour of the feral domestic fowl. *Animal Behav Monographs* **2**: 125–181.
- Parker GA (1970). Sperm competition and its evolutionary consequences in the insects. *Biol Rev Cambridge Philosoph Soc* **45**: 525–567.
- Parker GA (1998). Sperm competition and the evolution of ejaculates: towards a theory base. In: Birkhead TR, Møller AP (eds) *Sperm Competition and Sexual Selection*. Academic Press: London, pp 3–54.
- Pizzari T (1999). Reproductive Strategies of Feral Fowl. *Gallus gallus*. PhD Thesis, University of Sheffield.
- Pizzari T (2001). Indirect partner choice through manipulation of male behaviour by female fowl, *Gallus g. domesticus*. *Proc Royal Soc London Series B* **268**: 1–6.
- Pizzari, T (MS). Food, vigilance and sperm: the role of male direct benefits in the evolution of female preference in a polygamous bird. *Behav Ecol* (Submitted).
- Pizzari T, Birkhead TR (2000). Female feral fowl eject sperm of subdominant males. *Nature* **405**: 787–789.
- Pizzari T, Birkhead TR (2001). For whom does the hen cackle? The adaptive significance of post-oviposition cackling. *Animal Behav* **61**: 601–607.
- Pizzari T, Birkhead TR (2001). The sexually-selected sperm hypothesis: sex-biased inheritance and sexual antagonism. *Biolog Rev Cambridge Philosoph Soc* (In press).
- Pizzari T, Cornwallis CK, Birkhead TR (MS). The Coolidge effect: behaviour and sperm. *Proc Royal Soc Lond Series B* (Submitted).
- Pomiankowski A, Iwasa Y, Nee S (1991). The evolution of costly mating preferences. I. Fisher and biased mutation. *Evolution* **45**: 1422–1430.
- Qvarnstrom A, Griffith SC, Gustafsson L (2000). Male-male competition and parental care in collared flycatchers (*Ficedula albicollis*): an experiment controlling for differences in territory quality. *Proc Royal Soc London Series B* **267**: 2547–2552.
- Ruiz-Pesini E, Lapena AC, Diez-Sanchez C, Perez-Martos A, Montoya J, Alvarez E *et al* (2000). Human mtDNA haplogroups associated with high or reduced spermatozoa motility. *Am J Hum Genet* **67**: 682–696.
- Schütz KE, Jensen P (2001). Effects of resource allocation on behavioural strategies: a comparison of red junglefowl (*Gallus gallus*) and two domesticated breeds of poultry. *Ethology* **107**: 753–765.
- Sheldon BC (2000). Differential allocation: tests, mechanisms and implications. *Trends in Ecol Evol* **15**: 397–402.
- Thornhill R (1983). Female cryptic choice and its implications in the scorpionfly *Harpobittacus nigriceps*. *Am Naturalist* **122**: 765–788.
- Thornhill R (1988). The jungle fowl hen's cackle incites male competition. *Verhandlungen der Deutschen Zoologischen Gesellschaft* **81**: 145–154.
- Trivers RL (1972). Parental investment and sexual selection. In: Campbell B (ed) *Sexual Selection and the Descent of Man, 1871–1971*. Aldine-Atherton: Chicago, pp 136–179.
- Wiley RH, Poston J (1996). Indirect mate choice, competition for mates and coevolution of the sexes. *Evolution* **50**: 1371–1381.
- Wishart GJ (1987). Regulation of the length of the fertile period in the domestic fowl by numbers of oviducal spermatozoa, as reflected by those trapped in laid eggs. *J Reprod Fertil* **80**: 493–498.