Pre- and post-copulatory sexual selection in the fowl, *Gallus gallus*

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Abstract

The evolutionary goal of individuals is reproduction and sexual selection favours traits improving reproductive success. When males invest less than females in offspring, males have potentially a higher reproductive rate than females. This typically results in sex-specific reproductive strategies of male-male competition and female choice of mating partner. Under polyandry, sexual selection can continue after copulation as sperm competition and cryptic female choice. This thesis focuses on male and female pre- and post-copulatory reproductive strategies in the promiscuous red junglefowl, *Gallus gallus ssp.*, and its domestic subspecies the domestic fowl, *Gallus gallus domesticus*. Males impose high re-mating rates on females, which triggers female resistance in copulations. In addition, when sexual harassment increases, females re-mate at times of day when male mating propensity is lower, to avoid intense sexual harassment. Males allocate sperm supplies differentially according to (i) variation in female polyandry and own competitive ability, (ii) earlier sperm investment in a female, and (iii) female reproductive quality, signalled by female comb size. Males also perform ‘aspermic’ copulations (i.e. copulations with no semen transfer), which inhibit polyandry and in turn reduce sperm competition. In mating opportunities with relatives, males do not avoid inbreeding. However, females avoid inbreeding before copulation through kin recognition and after copulation by selecting against related males’ sperm. These results show that selection on males to re-mate at higher rates than females and copulate indiscriminately according to partner relatedness, trigger counteracting female responses, creating the potential for sexual conflict over fertilisation. Teasing apart pre- and post-copulatory strategies and the contribution of each sex therefore becomes crucial in order to understand the evolution of reproductive strategies and the mechanisms affecting paternity.

**Key words** cryptic female choice, *Gallus*, inbreeding avoidance, mate choice, sexual conflict, sexual selection, sperm competition
List of papers

The thesis is based on the following papers, which I will refer to in the text by their roman numbers (I-V):

I. **Løvlie, H., & T. Pizzari.** Sex in the morning or in the evening? Females adjust daily mating patterns to the intensity of sexual harassment. *American Naturalist*, in press.


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Introduction

Competing males and choosy females

Reproduction is the evolutionary goal of all individuals and the selection pressure caused by variation in individuals’ reproductive success is called sexual selection. Sexual selection is therefore a strong selection pressure promoting traits that confer a competitive advantage in reproduction. Reproductive success is increased through two main mechanisms; (i) competition for access to mating partners and (ii) choice of a sexual partner (Darwin 1871; Andersson 1994). Both sexes can be found as the competing and the choosy sex. Nevertheless, we typically find competing males and choosy females. Males produce a vast number of sperm, compared to the number of eggs a female produces and that are available to a male. As a result, males have potentially higher reproductive rates than females and their reproductive success is mainly constrained by the number of eggs that a male fertilises (e.g. Bateman 1948). Males are therefore sexually selected to increase their number of sexual partners, leading to male-male competition over females and mating opportunities (Darwin 1871; Andersson 1994). When copulation reduces number of females available for reproduction, for example during pregnancy, the ratio of males and females ready to mate (i.e. the operational sex ratio, Emlen & Oring 1977) becomes more male-biased and male competition for access to females increases further. Females potential reproductive rate, on the other hand, is mainly constrained by the number of eggs a female produces (Trivers 1972). Beyond securing sufficient amount of sperm to fertilise the whole clutch, multiple sexual partners (i.e. polyandry) often are unlikely to increase the number of zygotes produced by a female (Jennions & Petrie 2000). Because copulation may impose costs on females (e.g. Chapman et al. 1995; Gems & Riddle 1996; Stutt & Siva-Jothy 2001; Martin et al. 2004; Le Galliard et al. 2005), increased copulation rates may therefore increase these costs and, as a result, are not likely to contribute to increased female reproductive success. Instead of multiple copulations, a careful choice of a reproductive partner of high quality may increase female fitness (Anderson 1994). Benefits of choosing a high quality parner can be direct (i.e. the female her self benefits from her choice), or indirect (i.e. the female’s offspring benefit from the female’s choice). Indirect benefits are typically genetic benefits gained from mating with a partner with high genetic quality that increase the reproductive value of an offspring, either because the offspring inherit genes for enhanced survival or increased reproductive success (Fisher 1930; Zahavi 1975; Hamilton & Zuk 1982; Kokko et al. 2002), or offspring have increased fitness because the males genotype match the female genotype (Zeh & Zeh 1996; 1997; Jennions & Petrie 2000; Tregenza & Wedell 2000; Mays & Hill 2004; Neff & Pitcher 2005). Preference for a male whose genotype is compatible and matches the female genotype can increase offspring survival due to within-species heterozygosity. Increased survival of heterozygote offspring can be explained by dominance (i.e. when deleterious recessive alleles at specific loci are expressed in homozygote, but in heterozygote individuals are masked by the allele’s dominant counterpart) or overdominance (i.e. when a more genome-wise heterozygosis is beneficial and homozygosity reduces this benefit). A classic source of reduced heterozygosity is
inbreeding, reproduction between close relatives (Charlesworth & Charlesworth 1987; Keller & Waller 2002; but see Bench et al. 1994). Reproduction between related partners can therefore cause incompatibility and reduce offspring fitness. However, inbreeding can have positive effects on individuals’ fitness. Because relatives have more genes in common than unrelated individuals, allowing relatives to reproduce or gain additional reproductive opportunities by inbreeding can therefore increase inclusive fitness (Hamilton 1964). However, when the direct cost of inbreeding exceeds inclusive fitness gains, inbreeding should be avoided (Parker 1979; 2006; Lehmann & Perrin 2003; Kokko & Ots 2006).

Under the classic roles of competing males and choosy females, both sexes’ reproductive optima can often not be met, generating sexual conflict over mating and fertilisation. Manipulation of the other sex to increase own reproductive success and counteracting strategies to retain control of paternity may be triggered (Parker 1979; Arnqvist & Rowe 2005).

Post-copulatory mechanisms

Despite females’, compared to males’, reduced benefits from multiple mating it is more the rule than the exception in the animal kingdom that females are polyandrous (e.g. Birkhead & Møller 1998). Explanations for female polyandry have frequently been discussed (Arnqvist & Nilsson 2000; Jennions & Petrie 2000; Simmons 2005). Mainly in insects has polyandry been explained by females gaining direct benefits, and in vertebrate species females are typically polyandrous to obtain indirect benefits (Zeh & Zeh 1996; 1997; Jennions & Petrie 2000; Tregenza & Wedell 2000; Mays & Hill 2004; Neff & Pitcher 2005), or because males force female to copulate with them (Clutton-Brock & Parker 1995). Female polyandry nevertheless results in profound effects on sexual selection. When females receive sperm from multiple males sexual selection may continue after copulation, through the competition between ejaculates of two or more males over fertilisation of a female’s eggs (i.e. sperm competition, Parker 1970; 1998) and female bias in sperm utilisation in favour of ejaculates of certain males (i.e. cryptic female choice, Eberhard 1996; Birkhead 1998). Males’ uncertainty of paternity increases under polyandry, selecting males to avoid polyandry (e.g. Birkhead & Møller 1998). Males prevent females from mating with other males, for example by mate guarding (e.g. Birkhead 1979), application of copulatory plugs (e.g. Shine et al. 2000), removing earlier inseminated ejaculates (e.g. Gage 1992), inseminating large ejaculates (e.g. Cook & Wedell 1999) and/or inseminating specific seminal fluid proteins delaying female re-mating (e.g. Chapman et al. 1995; Woflner 2002). When males face sperm competition, the intuitive response is for a male to inseminate more sperm than his competitors in a female, in order to improve his fertilisation success. However, sperm production can be costly for males (Dewsbury 1982; e.g. Olsson et al. 1997) and male sperm supplies are limited (e.g. Birkhead 1991; Nakatsuru & Kramer 1982). Increased sperm investment in one female is therefore likely to reduce a male’s investment in other females and in turn constrain male reproductive success (e.g. Preston et al. 2001). In order to maximise reproductive success under sperm competition and with limited sperm supplies, males are therefore
predicted to allocate sperm supplies differentially to improve fertilisation success and according to the reproductive return of a copulation (Parker 1998; Wedell et al. 2002). Males should allocate sperm differentially according to the probability of facing sperm competition (i.e. the risk of sperm competition) by increasing the number of sperm inseminated when the risk of sperm competition increases (Parker et al. 1997; 1998; for review see Wedell et al. 2002). Males should also allocate sperm differentially according to the intensity of sperm competition (i.e. the number of males inseminating the same female) and counter-intuitively decrease the number of sperm invested into a female when the number of males inseminating the same female increases above two, to save sperm for less competitive mating opportunities (Parker et al. 1996; Parker 1998; e.g. Simmons & Kvarnemo 1997; Schaus & Sakaluk 2001; Pilastro et al. 2002). When males have an increased probability of obtaining future copulations they are selected to allocate sperm prudently and save sperm also for future copulations (Parker 1982; 1998; Reinhold et al. 2002). Males can also bias probability of fertilisation by targeting reproductively active females as copulation partners or copulate at times in a female’s oviposition cycle where insemination is most likely to result in fertilisation (Birkhead & Møller 1992; Birkhead & Pizzari 2002). Male choice of and higher investment in females of higher reproductive quality may also increase male reproductive success (Parker 1983; Reinhold et al. 2002; for review see Wedell et al. 2002).

Despite decreased control of paternity from the males’ point of view, polyandry may allow females to increase control of paternity, particularly in internally fertilising species where female pre-copulatory mate choice is constrained (e.g. Eberhard 1995; Pizzari & Birkhead 2000; Snow & Andrade 2005). Through cryptic female choice females may counteract male pre-copulatory strategies such as sexual coercion. The mechanisms of cryptic female choice is still scarcely studied, mainly due to the, at least in internal fertilisers, cryptic nature. However, cryptic female choice is known to cover a range of mechanisms (Eberhard 1996). From removal of spermatophores before sperm have been transferred (Eberhard 1996), ejection of ejaculates directly after copulation (e.g. Pizzari & Birkhead 2000; Wagner et al. 2004), further to involve differential sperm:female reproductive tract, or sperm:egg interactions biasing fertilisation success (e.g. Clark & Dell 2006). There is some evidence of cryptic female choice against aged sperm (Wagner et al. 2004), sperm of low-ranking males (Pizzari & Birkhead 2000), and several examples of cryptic female choice refer to bias in favour of genetically compatible partners (e.g. Olsson et al. 1996; Tregenza & Wedell 2002; Bretman et al. 2004; Simmons et al. 2006). Cryptic female choice may in these cases improve offspring quality and result in polyandry be adaptive for females (Reinhold 2002; for review see Jennions & Petrie 2000; Simmons 2005).

To conclude, the sexes have sex-specific reproductive optima and strategies to reach them. These strategies often differ, triggering counteracting responses and creating a potential for sexual conflict that, when females are polyandrous, may continue also after copulation. Knowledge of mechanisms of both sexes’ pre- and post-copulatory reproductive strategies are therefore crucial in order to understand what affects paternity.
Aim of this thesis

The aim of this thesis is to increase our understanding of cues and mechanisms affecting male and female reproductive strategies, both before and after copulation. Specifically, this thesis focused on:

- Male and female re-mating rates and daily re-mating patterns
- Male ability to allocate sperm strategically
- An adaptive explanation to the puzzling male behaviour ‘aspermic’ copulations
- Male pre- and post-copulatory responses to inbreeding
- Female pre- and post-copulatory inbreeding avoidance mechanisms

Study species

The wild red junglefowl and its domesticated relative

The red junglefowl, *Gallus gallus*, is native to Southeast Asia, and the subspecies *Gallus g. gallus*, the sole ancestor of all today’s domesticated chickens (Fumihito et al. 1994). Domestication probably occurred through one single domestication process and is dated about 8000 years back (Fumihito et al. 1994; 1996). Red junglefowl and domestic fowl interbreed, are morphologically and behaviourally similar (see ‘study populations’), and considered different subspecies. Throughout this thesis I will refer to both red junglefowl and domestic fowl as ‘fowl’. Both wild and feral fowl live in groups of 2-15 individuals, with a sex ratio of 1:1-1:4 males to females (Collias & Collias 1996; Sullivan 1991). Social hierarchies are sex-specific and typically linear. Social status predicts individuals’ behaviours and access to resources, for example are high-ranking males more vigilant than low-ranking males, and have increased access to females as mating partners (Banks 1956; McBride et al. 1969; Pizzari 2003).

Flocks of red junglefowl are small and relatively isolated in the wild. In addition, young birds seems to stay in their birth flocks even after sexual maturation (Collias & Collias 1996), and no difference in the movements of males and females between flocks been observed (Collias et al. 1966). This suggests that the fowl is prone to inbreeding. Consistent with this idea, 4% of matings observed in a captive, free-ranging red junglefowl population were between 1st order relatives (n=135, Collias & Collias 1996). In domestic fowl inbreeding reduces competitive ability (Craig & Bahruth 1965), mating frequency (Cheng et al. 1985), but also reduce hatchability of eggs and chick survival (Shoffner et al. 1953; Cheng et al. 1985; Abplanalp et al. 1992). Inbreeding is therefore likely to be costly. Nevertheless, no study has investigated potential inbreeding avoidance mechanisms in the fowl. Some species of galliforms are able to recognise kin (Petrie et al. 1999) and also avoid close kin as mating partners (Bateson 1982), suggesting the possibility of kin discrimination as a potential inbreeding avoidance mechanism also in the fowl.
Sexual selection in the fowl

The sexes follow the classic roles of competing males and choosy females. Males are larger and more ornamented than females, including having a larger comb (a fleshy head ornament, Johnsgard 1999). Both sexes are sexually promiscuous. Polyandry can be initiated by females (McBride et al. 1969; Ligon & Zwartjes 1995), but is mainly due to male coercion of females into copulation (McBride et al. 1969; Collias & Collias 1996; Etches 1996; Pizzari et al. 2002). Females can resist copulations directly, and are able to indirectly exert some control of copulation by uttering a distress call (Collias 1987) which attracts other males, and thus increases the likelihood that the copulation is disrupted (Pizzari 2001; Pizzari & Birkhead 2000). However, costs associated with copulation, like physical injuries (Pizzari 2001) and disrupted foraging, are likely to remain despite insemination may have been prevented.

The fowl has a bimodal daily copulation pattern with copulations occurring early in the morning and especially in the evening (McBride et al. 1969; Craig & Bhagwat 1974; Cheng & Burns 1988; Pizzari & Birkhead 2001) that can be explained by female daily oviposition pattern. Oviposition occurs in early and mid-half of the day (Christensen & Johnston 1977; Johnson 2000; Pizzari & Birkhead 2001). Copulations occurring few hours before and after oviposition have a decreased likelihood of resulting in fertilisation (Moore & Byerly 1942; Parker 1945; Johnston & Parker 1970; Christensen & Johnston 1977), selecting individuals to copulate outside the time of oviposition. The higher copulation peak in evenings can be explained by inseminations in evenings, being more likely to result in fertilisation than inseminations in the morning (Moore & Byerly 1942; Parker 1945; Christensen & Johnston 1977). Copulation in evenings may therefore be more efficient, selecting both sexes to preferentially copulate at this time of day.

Following insemination females store viable sperm in tens of thousands sperm storage tubules (SSTs) in the uterovaginal junction anterior to the vagina in the reproductive tract (Etches 1996). After insemination of a large ejaculate the production of fertile eggs declines rapidly after 10-14 days (Warren & Kilpatrick 1929; Etches 1996). Such prolonged sperm storage in addition to female polyandry creates potential for intense sperm competition (Pizzari et al. 2002). Male sperm supplies are limited and it takes 48 hours to replenish when exhausted (Parker et al. 1942). Males may therefore miss future mating opportunities if the amount of sperm inseminated into a female is increased, in attempt of improving probability of fertilisation. Male fowl are thus predicted to allocate sperm supplies differentially to be competitive in sperm competition and inseminate sperm to several females. Prolonged female sperm storage and polyandry also create the potential for cryptic female choice (Pizzari et al. 2002). One cryptic female choice mechanism is known in the fowl, whereby females eject ejaculates form subordinate males when these force females to copulate with them (Pizzari & Birkhead 2000). A study of domestic fowl detected a female bias in sperm utilisation after artificial insemination of males ejaculates (Birkhead et al. 2004). However, the cue and mechanism behind this bias remains unclear, nevertheless suggests the possibility of female influence of paternity occurring even later in the reproductive event, than differential sperm ejection.
Study populations

Tovetorp
In paper I-III, a population of an old Swedish game breed, ‘Gammal svensk dvärghöna’ (Gallus g. domesticus, Harrison 1987) kept at Tovetorp Zoological Research station (Stockholm University), was used. This population is random-bred under relaxed artificial selective pressures and birds marked with numbered leg-rings have been kept free-ranging in mixed-age, mixed-sex groups since the late 60’s. As a result, birds show strong similarities in morphology and behaviours to the red junglefowl (Schütz & Jensen 2001; Pizzari & Birkhead 2001).

Götala
In studies of responses to inbreeding, papers IV-V, a population of red junglefowl (Gallus gallus spp.) kept at SLU (Swedish University of Agricultural Sciences) in Skara, was used. This population originated from birds originally captured in Thailand and likely to be of the subspecies G. g. gallus or G. g. spadiceus (for further details, see Schütz & Jensen 2001). To know individuals’ relatedness, individually marked eggs from 18 pairs of sexually rested males and females for the first generation (used in paper IV-V) and 22 pairs for the second generation (used in paper V) were hatched in incubators. All chicks were marked at hatching with wing tags or neck labels. (Later were coloured leg-rings temporarily used to facilitate observations.) Siblings were randomly assigned to groups, resulting in socially familiar (i.e. that had been raised together) and unfamiliar individuals (i.e. that had not been raised together) both being full-sibs and unrelated individuals. Birds were kept in same-age, mixed-sex groups in indoor pens under standard housing condition according to Swedish welfare standards.
Some useful techniques

In addition to the promiscuous nature, the fowl is a suitable species for studies of pre- and post-copulatory sexual selection for at least two reasons. Firstly, birds are easily habituated to people and handling, enabling an experimental approach. Secondly, the use of certain techniques facilitates studies of otherwise cryptic post-copulatory mechanisms. The combination of those two resulted in techniques that have been useful for this thesis:

Controlled copulations

Copulations under controlled conditions are set up by first isolate a male and a female (at least physically) from the rest of the group. The female is held, initially facing the male to facilitate familiarisation between them. After a set amount of time, the female is turned around and presented to the male in a soliciting position allowing the male to copulate with her. The technique allows natural copulations to occur (in comparison to artificial insemination), but with more control of number of copulations and insemination success compared to free-ranging copulations. Insemination success can be quantified by looking for traces of semen around the female cloaca (paper III-V), or by the use of video cameras recording the copulation focusing on the female cloaca (paper V). Further can insemination be prevented by fitting the female with a harness covering her cloaca (paper III), facilitating collection of ejaculates allocated to the female (paper II, IV-VI). Volume of collected ejaculates were measured with a pipette, and if sperm numbers later were to be counted (according to Bakst & Cecil 1997) ejaculates were stored in 10% formalin.

Hydrolysis points on PVL

From the poultry industry it is known that in freshly laid eggs, the number of sperm that reached an ovum during the period of time available for fertilisation (i.e. following ovulation when the ovum is in the body cavity and upper infundibulum, Olsen & Neher 1948; Etches 1996) can be quantified by counting the number of hydrolysis points made by live sperm on the outer periviteline layer (PVL, i.e. the layer around the yolk) around the blastodisc of the egg (Wishart 1987; 1997). The pattern of variation in sperm numbers on eggs laid over successive days following an insemination provides an accurate measure of the amount of sperm a female store in her sperm storages (Brillard 1993). Sperm reaches the ovum 48 hours after fertilisation, and amount of hydrolysis points found on PVL of eggs typically drop over successive days as sperm is lost from SSTs (Lodge et al. 1971; Froman et al. 2002). Since the number of hydrolysis points observed depends on the area of PVL observed, the magnifications of the microscopes used are likely to give different number of observed and counted sperm. In this thesis, two different microscopes have been used, explaining why sperm numbers counted in paper IV are higher than in paper V.
Results

Male strategies
As predicted from the lesser investment in offspring than females, males re-mate at higher rates than females (paper I), and copulate with both related and unrelated partners (paper IV). In addition, as predicted from their limited sperm supplies and faced sperm competition, males allocate sperm supplies differentially according to (i) earlier sperm investment in a female (paper I-II), (ii) variation in polyandry and own competitive ability (paper II) and (ii) female reproductive quality (paper II, IV).

Copulation rates and copulation history
Male and female re-mating rates were studied in free-ranging groups of fowl with three different sex ratios (2:8, 4:6, 6:4, males to females) and over two time scales; cumulative time of exposure to the opposite sex following sexual rest (i.e. days), and time of day (i.e. mornings and evenings). In all sex ratios males adopted much higher re-mating rates than females and initiated more than 90% of all observed copulation attempts (n=3568). However, male re-mating rates were reduced after the first day after sexual rest (paper I, figure 1).

![Graph showing male and female remating rates across days.](image)

Figure 1. Male and female re-mating rates in free-ranging groups, across days. Re-mating rates (number of copulations initiated by an individual, per hour observed) were sex-specific. Males (empty) initiated more copulations than females (filled) and initiated more copulations on the first day after sexual rest (day: F_{3,752}=9.46, p≤0.0001). Females solicited fewer copulations over successive days (day: F_{3,748}=59.46, p≤0.0001, for further details and statistics, see paper I). Columns represent means, bars represent standard error.

A reduction in male sexual interest with increased exposure to and sexual familiarity with a female can be explained by increased cumulative number of inseminations and/or reduced male sexual activity because of exhausted sperm supplies. Because only pre-copulatory behaviours were observed in this study, these two explanations can not be disentangled. However, in paper II, male sexual interest and also sperm allocation over successive copulations across females was quantified by Cornwallis and Pizzari, showing that males lost sexual interest in a female with increased number...
of copulations with her, after controlling for sperm depletion. One male at the time was allowed to copulate with a female until he lost interest in her, whereby the male was presented a new female. Male propensity to copulate with a particular female declined with time, but was renewed by replacing the female with a new female. Similarly, sperm numbers in males’ ejaculates declined over successive copulations with a female, and even resulted in copulations without semen transfer at all. However, sperm numbers were restored when the male copulated with a new female, to once again decline over successive copulations (paper II, figure 2).

Figure 2. Relative sperm investment over successive copulations and across females. Relative sperm investment of male fowl declined over successive inseminations (circles) with the same female (circles in same colour) and was renewed by the presence of a new female. Males tend to invest more sperm in the first ejaculate with a new female than in the last ejaculate with the familiar female (for statistics and further details, see paper II, figure 3). Circles represent means, bars represent standard error.

This copulation pattern show two things, (i) that males allocate sperm differentially over successive copulations across females, and (ii) that the lack of sperm in ejaculates late in the copulation sequence with a female is not due to sperm depletion. Firstly, males allocate sperm reserves in a way saving sperm for future copulation opportunities. Sæther and co-workers (2001) showed a similar male behaviour in the sense that males did not re-mate with females they already had mated with. This suggests that males use sexual familiarity with a female as a cue to reduce investment in her to save sperm for future copulations (Wedell et al. 2002). Secondly, the observed copulations without any semen transfer (i.e. ‘aspermic’ copulations, also observed in other taxa: Parker et al. 1942; Dewsbury 1972; Adkins 1974; Birkhead et al. 1988; Birkhead 1991; Hunter et al. 1996; Westneat et al. 1998; Baker et al. 2001; Gronlund et al. 2002), have typically been explained by males being sexually inept or sperm depleted. However, the pattern of aspermic copulation observed in the fowl suggests that these copulations may have a function since performed by sexually mature males that were not sperm depleted. The effect aspermic copulations had on female polyandry therefore was investigated. In paper III, females were exposed to
aspermic copulations, simulated by fitting females temporarily with a harness and copulations occurring under controlled conditions. Females exposed to aspermic copulations responded in the same way as inseminated females, thus with reduced propensity to re-mate compared to non-mated females. The reduction in female re-mating propensity was therefore explained by mounting per se. The observed reduced female re-mating propensity can be explained by mounting manipulating females to perceive that they have received more sperm than they have. Exposure to aspermic copulations may in turn discourage females from re-mating, if copulations are costly to females. Female response to mounting lasted for about two days, long enough to translate into females storing fewer sperm from a new male (paper III, figure 3).

Figure 3. Aspermic copulations and sperm competition. Standardised number of hydrolysis points counted on the periviteline layer of eggs laid of females experimentally exposed to natural copulations (open circles), aspermic copulations (filled circles) or no exposure to copulations (diamonds), when later exposed to a new male free-ranging for two days. Fewer sperm reaches the eggs of females that were exposed to aspermic copulations than eggs produced by females exposed to any other experimental treatment, indicating that a reduction in female polyandry was translated into a short-term reduction in sperm competition intensity (for statistics and further details, see paper III, figure 2). Symbols represent means, bars represent standard error.

Some days reduction in sperm competition (figure 3) may be enough time to improve a male’s chances of fertilisation, at least for an egg or two of a females clutch. In the fowl, no pre-copulatory tactic used by males to prevent female polyandry is known, except from males interrupting each others copulations (Pizzari 2001). Males copulating without semen could be a male strategy to reduce female re-mating propensity temporarily, and at the same time allow males to save sperm for future matings. Under what conditions and to what extent male fowl perform aspermic copulations (but from with described above and shown in figure 2) and also how taxonomically widespread it is, is not known. However, in some birds, males typically mate-guard females to prevent polyandry (e.g. Birkhead 1979), and in species that do not mate guard, high copulation frequencies are often observed (≥20 copulations/clutch/female), suggested to be an alternative strategy to mate guarding.
In species with high copulation frequencies and frequently observed ‘failing’ of semen transfer in copulations may therefore be interesting to further study to test this idea. In addition, inhibiting female polyandry by mounting may be cheaper than other observed specialised male copulatory traits and seminal products produced by males to reduce female promiscuity (e.g. Chapman et al. 1995).

Further investigating re-mating rates over time of day showed that males preferentially initiated copulations in evenings, when a copulation is more likely to result in fertilisation, compared to mornings (Moore & Byerly 1942; Parker 1945; Christensen & Johnston 1977). Males were also more rapidly attracted to a soliciting female and closer to females at this time of day (paper I). Timing copulations to this time may therefore increase probability of fertilisation (Birkhead & Møller 1992; e.g. Preston et al. 2003). However, sperm competition is likely to be particularly intense in evenings due increased mating propensity by all males at this time of day (paper I). This in turn may reduce the probability of fertilisation in evenings, selecting males to be able to allocate sperm supplies also according to the sperm competition faced.

**Sperm allocation and rivalry**

High re-mating rates results in female fowl typically being polyandrous and an intense sperm competition (Pizzari et al. 2002). Under these conditions males are predicted to allocate sperm supplies differentially according to the risk and intensity of sperm competition (Parker 1998). In paper II, male perception of female polyandry was altered by housing males alone, with one or with three competitors (paper II). When allowed to copulate until lost sexual interest in a single female, males allocated their sperm supplies differentially both according to experimental treatment, but also according to their own competitive ability (i.e. social status). In the absence of any competitors, males minimised their sperm investment. This is a response predicted theoretically because the chance of fertilisation is higher than when facing sperm competition (Parker et al. 1997; Parker 1998; for review see Wedell et al. 2002). As the number of competitors increased from one to three, dominant males increased their sperm investment, but subdominant males maximised their investment in the presence of just one competitor. Dominant males have greater access to females and control over copulations (Cheng & Burns 1988; Pizzari 2001), and the presence of other males may represent an increased risk of another male inseminating the same female (but not necessarily an increased number of males inseminating this female). Subdominant males cannot prevent females from copulation with other males (Pizzari 2001) and the presence of other males is likely to result in more intense sperm competition, predicting males to reduce their sperm investment when competition increases above one competitor. The reduction in male sperm allocation with increased sperm competition intensity is theoretically predicted (Parker et al. 1996; Parker 1998; e.g. Simmons & Kvarnemo 1997; Pilastro et al. 2002) and may allow males to conserve sperm for future less competitive reproductive opportunities. Cues like number of rivals present and also own social status therefore provides information on the risk and intensity of sperm competition enabling males to allocate sperm strategically.
Figure 4. Differential sperm allocation and female polyandry. Dominant males (filled columns) increased relative sperm investment as the number of competitors increased, whereas subordinate males (open columns) decreased their sperm investment when the number of competitors increased above one (for statistics and further details, see paper II, figure 1). Columns represent means, bars represent standard error.

Sperm allocation and female quality
With limited sperm supplies and variation in female quality, males should inseminate more sperm to females of higher quality (Parker 1983; 1998; Reinhold et al. 2002; e.g. Gage & Barnard 1996; Byrne & Rice 2006). In paper II it was shown that females with larger combs produced larger eggs with larger yolks, thus that female comb size signal maternal quality. Males used this cue for female quality and preferred females with larger combs both in free-ranging groups and in a two-choice situation. Both socially dominant and subordinate males also allocated more sperm to females with larger combs. However, dominant males biased their sperm allocation in favour of large-combed females more distinct, which in turn may bias paternity of high quality offspring in favour of dominant males.

Further, female quality can vary due to genetic relatedness with the male. This is because mating with relatives on one hand can be beneficial and increase an individual’s inclusive fitness, but on the other hand may reduce offspring fitness due to inbreeding depression (Parker 1979; 2006; Kokko & Ots 2006). When males were presented with females related and unrelated to the male, males’ probability to copulate with either of the females did not differ (paper IV). Males showed a tendency to initiate copulations faster with unrelated females, and also to allocate more sperm to sisters. Differentiation between related and unrelated females suggests that males are able to recognise kin. On the other hand, the effect of genetic relatedness was influenced by social familiarity, in other words whether females had been raised with the male or not, and further studies are needed to disentangle the two effects. However, males did not avoid inbreeding. In species with little paternal care, males are predicted to have higher inbreeding tolerance than females (for female response to...
inbreeding, see below) and particularly high inbreeding tolerance if access to females is constrained. Both criteria were fulfilled in paper IV since males were only allowed to copulate with one female at the time, showing a predicted male response, also to inbreeding (Parker 1979; 2006; Kokko & Ots 2006).

To summarise, males have high re-mating rates and copulate with females independent of their relatedness with the male. Males also target fecund females and copulation at time of day increasing the potential reproductive value of a copulation. Males show plastic behaviours as a response to the selective pressures created by limited sperm supplies and sperm competition. Male differential sperm allocation is triggered by several cues, thus males allocate sperm reserves in a complex manner. The mechanism of male sperm allocation is still unknown, but it is clear that it regulates sperm numbers in an ejaculate within a short time scale, because from the last copulation with one female and to the first copulation with a novel female only about 10 minutes elapsed (paper II).
Female strategies
Females’ greater investment in offspring than males’, and the high number of male-initiated copulations females were exposed to, resulted in flexible daily remating patterns to avoid intense sexual harassment, female resistance in copulations when the cumulative number of copulations increased (paper I) and when females were currently not laying (paper III, V). In addition, as predicted from females bearing a greater cost of an inbred offspring, females attempted to avoid inbreeding both before and after copulation (paper IV-V).

Responses to sexual harassment
In free-ranging groups of fowl, females had their highest re-mating propensity when sperm storages were empty (i.e. after sexual rest), explaining female solicitation by ensuring sperm for fertilisation (paper I). Consistent with this idea, female re-mating propensity declined when the cumulative number of copulations increased (paper I, III, V, figure 5) and in addition, non-laying females (i.e. females that did not lay any eggs within at least 10 days following behavioural observations) with no need for sperm, solicited fewer copulations than laying females (paper I, III, V). The general pattern of female mating behaviours was that when female solicitation rate declined, female resistance in male-initiated copulations increased (paper I, III, V). Already in female-biased groups (2:8 males to females) were female resistance triggered, concluding that the optimal female mating rate is relatively low and much lower than males’ (paper I, figure 1). In the fowl, male-initiated re-mating rates increased due to increased number of males in a group, and not due to individual male re-mating rates increased. An exposure to higher re-mating rates for females in more male-biased sex ratios is intuitive and often shown (e.g. Holland & Rice 1999; Martin & Hosken 2004; Crudgington et al. 2005; Kraaijeveld et al. 2005; Le Galliard et al. 2005; Wigby & Chapman 2005; Head & Brooks 2006). Nevertheless, this variation in group sex ratio not only triggered female resistance in the fowl, but also shaped female daily remating patterns. In the most female-biased groups (2:8 males to females), both sexes initiated more copulations in evenings, the time of day inseminations are most likely to result in fertilisation (Moore & Byerly 1942; Parker 1945; Christensen & Johnston 1977). While males, independent of groups’ sex ratios, continued to initiate copulations mainly in evenings, females on the other hand shifted the time of solicitation from evenings to mornings when sex ratios became more male-biased (paper I, figure 5). This pattern of female daily re-mating can be explained by variation in sexual harassment intensity. In male-biased groups, females are not only exposed to more male-initiated mating, but males were also closer to females and more rapidly attracted to soliciting females, particularly in evenings. By the use of daily variation in male re-mating propensity and distribution patterns of populations, females plastically adjusted the timing of solicitation to the level of sexual harassment of a group. The high rate of male-initiated copulations and also male sexual coercion may constrain female pre-copulatory mate choice in evenings. Prolonged sperm storage may therefore enable females to reduce the number of copulations needed to both ensure enough sperm for fertilisation, but also allow flexibility in when during the day to initiate a copulation. Female mating behaviours may therefore give an
explanation for the observed copulation peak also in mornings in the fowl. In addition, this behavioural pattern may be an adaptive response if females retain pre-copulatory control of copulation and/or reduce costs associated with copulation by adopting it.

Figure 5. Female daily re-mating pattern over time of day and sex ratios. Females solicited more copulations (number of solicitations per female, over minutes observed) in the morning (empty columns) than in the evening (filled columns) in the most female-biased groups (i.e. 2:8 males to females), and reversed this trend in male-biased groups (i.e. 6:4 males to females), while there was no difference in solicitation rate in groups of the intermediate sex ratio (for statistics and further details, see paper I, figure 4). Columns represent means, bars represent standard error.

**Inbreeding avoidance**

Contrary to males, females avoided inbreeding, and did so both before and after copulation (paper IV, V). In groups of one male, his two sisters and two females unrelated to the male, females related to him initiated fewer copulations than unrelated females (paper V). Related females also resisted more copulations from the focal male (paper V). The difference in related and unrelated females’ responses to the focal male confirms the suggestion from paper IV that the fowl discriminate between kin and non-kin, independent of social familiarity (females, paper V, males, H. Løvlie, unpublished data). Kin recognition therefore is based on a genetic cue and not on earlier social familiarity (Holmes & Sherman 1982; Holmes 1986). The evolution of a kin recognition mechanism independent of familiarity makes sense in the light of the promiscuous mating system of the species, where social familiarity, for example among offspring, does not necessarily translate into genetic relatedness (similarly shown in e.g. Hain & Neff 2006). A reliable cue used in kin recognition should be a direct predictor of genetic relatedness, for example individual variation in genes of the major histocompatibility complex (MHC, Klein 1986). The MHC is a highly polymorphic gene complex important in the vertebrate immune system (Hughes and Yeager 1998). Due to the high polymorphism of the MHC, MHC-similarity of two individuals is likely to co-vary with their genetic relatedness and may in turn enable kin recognition (Penn 2002; Zelano and Edwards 2002). Confirming the idea, variation in MHC has been implicated in kin discrimination in several vertebrate species, often mediated by olfactory cues (e.g. Brown and Eklund 1994; Olsen et al 1998; Penn and
Potts 1999; Rajakaruna et al. 2006). Studies of variation in MHC affecting mate choice in birds (von Schantz et al. 1996; Freeman-Gallant et al. 2003; Ekblom et al. 2004; Richardson et al. 2004; Bonneaud et al. 2006) and also that birds respond to olfactory cues (for reviewed see Zelano and Edwards 2002; domestic fowl, Jones & Roper 1997). Together, this offers the possibility that olfaction may be more important in the fowl than earlier assumed and also suggests a link between olfaction, MHC and kin recognition.

Female red junglefowl also avoided inbreeding after copulation. After copulations in free-ranging groups (paper V), more hydrolysis points were found on eggs laid by females unrelated to the focal male, compared to counts on eggs produced by the male’s sisters (figure 6a). A similar pattern in bias of unrelated males’ sperm was found also in paper IV. However, in paper IV female pre-copulatory behaviours was controlled for (i.e. copulations occurred under controlled conditions). This suggests that the pattern of female sperm utilisation found after free-ranging copulations may not solely be due to female pre-copulatory behaviours despite the intuitive explanation it offers. Replicating the experiment from paper IV confirmed the sperm pattern, concluding that females utilised less sperm from a related male independent of female pre-copulatory behaviours, and neither explained by males’ differential sperm allocation and allocation of more sperm to unrelated females (paper IV), nor by female differential ejaculate ejection (paper V). This suggests another female-driven cryptic mechanism other than differential sperm ejection to occur. The bias in sperm utilisation explained by genetic relatedness was heavily reduced when ejaculates of males were inseminated artificially. This suggests that the mechanism causing the bias is either occurring or triggered early in the female reproductive tract, and/or is triggered by a pre-copulatory cue. After insemination, typically only a small proportion of an ejaculate is stored in SSTs (Brillard & Artoine 1990; Bakst et al. 1994). Potential female-driven mechanisms biasing sperm utilisation could therefore be by affecting sperm transport to SSTs, for example by a vaginal barrier selecting against certain males’ sperm, however could also occur by biasing transport from sperm storages to the infundibulum where fertilisation occurs (Olsen & Neher 1948; Etches 1996).

To summarise, females counteract male reproductive strategies by adopting flexible pre-copulatory behaviours explained by variation in - and avoidance of - intense sexual harassment. Females also avoid inbreeding both before and after copulations, the latter by a cryptic female choice still not known and encouraging further investigation. The prolonged female sperm storage that generates the potential for sperm competition in the species may also be the trait enabling the above described female behaviours. Reducing the number of inseminations needed to ensure fertilisation, in combination with avoidance of solicitation at the daily peak in sexual harassment intensity may make it possible for females to seize considerable more control of mate choice and perhaps reduce costs of high re-mating rates (paper I). In addition, female sperm storage enables a prolonged time for female influence on post-copulatory mechanisms (paper IV-V) likely to reduce risk of inbreeding.
Figure 6. Patterns of female sperm utilisation. (A) After free-ranging copulation with the focal male did unrelated females (empty points, dashed line) retain more sperm (number of sperm counted on the perivitel line layer of eggs laid after insemination) in the first days compared to related females (filled points, solid line). (B) Females retained more sperm after copulations with unrelated (empty points, dashed line) compared to related males (filled points, solid line). (C) Most variation in female sperm utilisation earlier explained by relatedness disappeared after artificial insemination (unrelated: empty points, dashed line; related females: filled points, solid line; for statistics and further details, see paper V, figure 1 and 3a,b). Points represent means, bars represent standard error.
Discussion

When interests differ

Sexual reproduction creates the possibility for one sex to manipulate the other, that in turn triggers counteracting responses and creates a potential for sexual conflict over mating and fertilisation (Parker 1979; 2006). In the fowl the sexes’ skewed investment in offspring results in sex-specific reproductive strategies and reproductive interests that may differ for mating rates (paper I), female sperm limitation (paper I, II, III) and inbreeding (paper IV, V).

Male fowl are selected to re-mate at higher rates than females, and due to sexual dimorphism males are able to force females to copulate (McBride et al. 1969; Collias & Collias 1996; Etches 1996; Pizzari et al. 2002). Already in groups with highly female-biased sex ratios (2:8 males to females) males imposed high enough mating rates on females to trigger female resistance in copulations (paper I). However, female costs of high re-mating rates were not directly measured. Although, female counteracting responses triggered by male behaviours suggest the occurrence of inter-sexual conflict (Arnqvist & Rowe 2005). Confirming this idea, non-laying females resist more than laying females when exposed to male-initiating copulations. “Non-laying” was here defined as when a female was not laying any eggs in at least 10 days following observations. Non-laying females therefore did not gain any benefits from copulation. The observed resistance is therefore a measure of female unwillingness to engage in copulations, suggesting there to be costs for females associating with copulations, and further a sexual conflict over re-mating.

Few studies have focused on the reproductive value of a copulation changing over time, but rather have focused on sexual conflict over mating rates (but see Parker 1970b). In groups with a male-biased sex ratio (6:4 males to females) females were triggered to re-mate in mornings instead of evenings due to the intense sexual harassment in evenings (paper I, figure 5). Copulations in mornings, compared to evenings, are known to be less likely to result in fertilisation (Moore & Byerly 1942; Parker 1945; Christensen & Johnston 1977), which could create a sexual conflict over female sperm limitation (Wedell et al. 2002). Similarly, male extreme sperm allocation and performance of aspermic copulations could result in female sperm limitation (Wedell et al. 2002). Nevertheless, infertility is not very likely to be a cost females bear from copulating in mornings nor through exposure to aspermic copulations. This is because the long female sperm storage may allow females in male-biased groups (paper I) to solicit copulations in mornings ensuring storage of sperm from the preferred males, and thus reduce the need for involving in copulations in evenings when sexual harassment is higher. For females exposed to aspermic copulations (paper III), the prolonged sperm storage may reduce the risk of sperm depletion for the few days polyandry was inhibited. The cost that triggers female counteracting responses is therefore more likely to be the cost of engaging in copulations per se, or from being sexually harassed and not sperm limitation. Alternatively, sperm limitation has been a cost to females earlier in the arms race between males and females over control of
paternity and in turn already triggered polyandry (Pizzari 2002) and prolonged sperm storage as the counteracting responses.

Genetic relatedness between partners also created a potential for conflict. Males copulate indiscriminately according to partner relatedness, while females attempted to avoid inbreeding both before and after copulation (paper IV, V). As a result, males sexually coerce sisters to copulate, triggering females resisted directly (paper V). The occurrence of also a post-copulatory inbreeding avoidance mechanism suggests that female pre-copulatory resistance in copulation is circumvented and males are able to constrain female pre-copulatory mate choice. A cryptic female choice then may enable females to counteract male coercion and reduce the risk of inbreeding. This in turn may select for males insemiinating more sperm into sisters to overcome female selection against related males’ sperm, continuing the conflict over fertilisation (paper IV). Counteracting responses may thus continue as an arms race between the sexes, where both sexes is an important part of the selective environment of the other sex (Moore & Pizzari 2005; e.g. paper VI), counteracting responses and sexual conflict therefore needs to considered in studies to fully understand the evolution of reproductive strategies.

Behaviours as a predictor of post-copulatory processes

This thesis also points out the fact that copulation can be a poor predictor of fertilisation. Typically (particularly in studies of wild species), studies of variation in paternity observe social pairing, or copulation success, and assign offspring paternity to individuals in the population (e.g. birds, Brooker et al. 1990; Blomqvist et al 2002; Forester et al 2003; Hansson et al. 2004). By adopting such an approach we may get detailed information on the outcome of a reproductive event, but it leaves a gap between the observed social pairing and paternity. In other words, we may gain no or very limited information on the mechanisms affecting paternity. In the fowl, after a successful copulation there is about two weeks of potential competition and choice that can affect paternity (Parker et al. 1942; Etches 1996; Pizzari et al. 2002). But by the use of an experimental approach and the possibility to quantify male ejaculate allocation and female sperm utilisation, it has been possible to study and disentangle pre- and post-copulatory effects and mechanism that in other species are impossible. And as a result this thesis has contributed to filling some of this gap between copulation and paternity. The thesis shows that male sperm allocation in the fowl is triggered by several different cues (e.g. sexual familiarity with a female, female quality, male social status, female polyandry level, paper II; genetic relatedness, paper IV) resulting in complex predictions about male sperm allocation pattern and further of paternity. Similarly complex, female selection against males after mating is also triggered by several cues (e.g. male social status, Pizzari & Birkhead 2000; genetic relatedness and social familiarity, paper V) and by several types of cryptic female choice (paper V). Further, the thesis shows that pre- and post-copulatory behaviours can mirror each other, for example when females avoid inbreeding both before and after copulations (paper IV-V). However, pre- and post-copulatory behaviours may in other cases not coincide, which is true with aspermic copulations where behaviourally
successful copulations do not result in semen transfer (paper II-III). The later could lead to misleading predictions of paternity made from an observed mating (Garcia-Gonzalez 2004). Further complicating predictions of paternity is that just because one mechanism is known to occur, there may still be other mechanisms taking place, even within the same individual and triggered by the same cue. This is the case with both pre- and post-copulatory inbreeding avoidance in female fowl, and also the occurrence of several cryptic female choice mechanisms (paper V). As a result, behavioural observations therefore may tell little or nothing about the post-copulatory mechanisms affecting paternity, but only shows the pre-copulatory outcome of male and female reproductive strategies. To further increase our understanding of post-copulatory mechanisms more studies, enabling the teasing apart of pre- and post-copulatory male and female-driven strategies, are therefore needed.

Conclusions
To conclude, this thesis shows that skewed investment in offspring by the sexes results in sex-specific reproductive strategies in the fowl. Males have higher mating rates than females and also copulate indiscriminately according to partner relatedness. This in turn triggers female counteracting responses and flexible re-mating behaviour which may reduce costs of high mating rates and inbred offspring, the later by adopting both pre- and post-copulatory inbreeding avoidance. Female fowl are polyandrous, creating the potential for sperm competition and cryptic female choice. Males respond to the selection pressure caused by sperm competition and limited sperm supplies by allocating sperm supplies differentially in a complex and plastic manner. The patterns of male differential sperm allocation are predicted to – at least theoretically – improve the reproductive return of an insemination and increase the probability of fertilisation. Females exert post-copulatory strategies likely to enable females to retain control of paternity, by both ejecting ejaculates and biasing sperm utilisation even at a later stage in a reproductive event. Further more, the sexes’ different reproductive strategies create a potential for sexual conflict over re-mating rates and inbreeding. Through the fruitful mix of an experimental approach together with behavioural observations and the possibility to quantify physiological processes, the results of this thesis expose cues and mechanisms affecting pre- and post-copulatory processes and determine whether they are male or female driven. As a result, by combining the possible ways male and female pre- and post-copulatory strategies can affect paternity, it becomes challenging – if at all possible – to predict paternity by observing only behaviours. Nevertheless, the fowl is now one of the most well-studied species regarding pre- and post-copulatory sexual selection, contributing significantly to our general understanding of sexual selection and mechanisms affecting paternity, and the results obtained from this thesis will only enhance that position.
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När hanar slåss och honor väljer

Hönan å andra sidan har ju potentiellt möjligheten att gynnas av spermie konkurrens genom att flera hanars spermier befinner sig i henne vid samma tid, och då ”besluta” om vem som befruktar ägget då befruktningen sker inuti henne. Detta kan potentiellt ske på en rad olika sätt från att honan gör sig av med ejakulatet direkt efter inseminering (”sperm ejection”), att honan påverkar transporten och lagringen av spermier, eller att ägget i sig påverkar vilken spermie som slutligen släpps igenom och befruktar det. Ett kryptisk honval behöver därför inte vara ett aktivt val direkt styrt av honan, men att det är honan som på något sätt ligger bakom det vissa hanars spermier gynnas före andras.


Höns och djungelhöns


Lösaktiga höns

Hönan tar ensam hand om kycklingarna, vilket leder till klassiska könsroller med tuppar som släss och hönor som väljer. Höns är promiskuösa mestadels för att tuppar inte låter hönorna vara i fred. Detta i kombination med att hönor sparar spermier i tion-tusentals lagringsorg in i sitt reproduktiva system i upp till två veckor efter en parning (vilket är länge jämfört med däggdjur som ofta sparar spermier under några dagar bara), leder till intensiv spermiekonkurrens och möjligheter till kryptiskt honval utöver ”sperm ejection”.

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Höns och inavel

Syftet med avhandlingen
Syftet med avhandlingen är att öka vår kunskap och förståelse om hur sexuell selektionen verkar och vad som påverkar individers tillgång till parningspartners. Jag har använt höns som modellart, och hos dessa studerat båda köns reproduktiva strategier både före och efter parning.
Mera specifikt har jag studerat:
- Hanars och honors dagliga parningsmönster och parningsfrekvenser
- Hanars förmåga att allokera sina spermieresurser på ett ekonomisk sätt
- Det konstiga beteendet att hanar ”fejkar” parningar d.v.s. inte inseminerar spermier vid en parning
- Hanars respons mot inavel, före och efter parning
- Honors olika sätt att förhindra inavel, både före och efter parning

Sexuella trakasserier (artikel I)
Hanar oftare än honor ökar sin fortplantningsframgång genom att ha flera partners. Detta kan leda till att hanar utsätter honor för sexuella trakasserier och -från honans synpunkt- överflödig eller försvunnen parningsinviter. Detta resulterar ofta i att honan blir mer avtänd och ännu mindre intresserad. Hos hönsen utför tuppar över 90% av alla parningsinviter, och oftast på kvällen efter det att hönorna har lagt sina ägg och då det är större chans att en parning leder till befruktning. I grupper med mestadels honor utsätts hönorna inte för så intensiva trakasserier, och då blir också hönorna mer intresserade av parningar på kvällen. Men i grupper med många tuppar blir uppmärksamheten ökade intresse för mycket för hönorna, och hönorna väljer istället att initiera parningar på morgonen när tupparna är mindre intresserad och också mer uttropna. Hönorna väljer därför på ett flexibelt sätt sina tillfällen när på dagen de initierar parningar beroende på hur mycket sexuella trakasserier de utsätts för.
Tuppars spermieekonomi (artikel II)


Fejkade parningar (artikel III)

Trots att det evolutionära målet för en individ är att for planta sig är det känt i djurvärlden – från insekter till däggdjur – att hanar ibland parar sig med en hona utan att inseminera några spermier. Även fullt könsmogna, erfarna tuppar med fulla spermieresurser gör detta, typiskt efter det att de redan har parat sig och inseminerat spermier i en höna. Genom att utsätta hönor antingen för parningar där tuppar inseminerade sperma, eller ”fejkade” parningar (genom att tillfälligt ha ”rumpskyddet” på dessa hönor i annars naturliga parningar), kunde hönorans respons på detta beteende studeras. Och det konstiga är att hönor som utsattes för fejkade parningar var lika ointresserade av att para sig med nya tuppar som de hönor som också mottog sperma i parningarna, båda i motsats till de hönor som inte utsattes för endera av parningarna. Reduktioner i hönorans promiskuitet efter parning har hittats också hos andra arter, men effekten har då berott på att hanarna har inseminerat stora mängder sperma, inseminerat sofistikerade produkter i sädessvätnkan, uppvisat särskilda beteenden eller på annat sätt investerat kostsamt i att minska honans lösaktighet. Fejkade parningar å andra sidan kan vara ett mycket enklare sätt att reducera hönorans promiskuitet. Genom att fejka parningar efter att redan ha inseminerat sperma till en höna kan tuppar kämpa om sitt faderskap, samtidigt som de sparar sperma till andra hönor, och det tillsynes meningslösa beteendet får en mening.
.Syskonbråk om syskonkärlek (artikel IV)

.Hur hönor förhindrar inavel (artikel V)
Konklusion