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Sex-specific, counteracting responses to inbreeding in a bird

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Inbreeding often depresses offspring fitness. Because females invest more than males in a reproductive event, inbreeding is expected to be more costly to mothers than fathers, creating a divergence between the reproductive interests of each sex and promoting sex-specific inbreeding strategies. Males and females may bias the probability of inbreeding by selecting copulation partners, and, in sexually promiscuous species, through male strategic sperm investment in different females and female selection of the sperm of different males. However, these processes are often difficult to study, and the way that different male and female strategies interact to determine inbreeding remains poorly understood. Here we demonstrate sex-specific, counteracting responses to inbreeding in the promiscuous red junglefowl, *Gallus gallus*. First, a male was just as likely to copulate with his full-sib sister as with an unrelated female. In addition, males displayed a tendency to: (i) initiate copulation faster when exposed to an unrelated female than when exposed to a sister, and (ii) inseminate more sperm into sisters than into unrelated females. Second, females retained fewer sperm following insemination by brothers, thus reducing the risk of inbreeding and counteracting male inbreeding strategies.

Keywords: cryptic female choice; genetic compatibility; incest; kin; sperm allocation; sexual conflict

1. INTRODUCTION

Homozygosity can depress fitness as a result of the expression of deleterious recessive alleles and loss of heterozygote advantage (Charlesworth & Charlesworth 1987; Lynch & Walsh 1998), and both sexes should prefer unrelated partners to avoid inbreeding (Pusey & Wolf 1996; Meagher *et al.* 2000; Kruuk *et al.* 2002; Tregenza & Wedell 2002; Reid *et al.* 2003). However, males have higher potential reproductive rates than females (Trivers 1972) and this may create inter-sexual conflict over inbreeding in promiscuous systems where individuals are exposed to multiple copulation opportunities (Parker 1979, 1983; Smith 1979; Perrin & Mazalov 2000). This conflict arises for two reasons. First, inbreeding reduces offspring viability so that relative to non-inbred offspring, the viability of inbred offspring, is $1-\delta$, where δ is the cost of inbreeding. Therefore, a male will increase his reproductive success by op (o is the number of eggs fertilized and p is the probability of embryo survival) for each unrelated female, and by $o(p-\delta)$ for each related female that he inseminates. This selects males to copulate preferentially with unrelated partners, and also to inseminate additional related partners whenever (i) unrelated females are unavailable, and (ii) the benefits of additional reproductive success that males gain through inbreeding ($o(p-\delta)$) exceed the inclusive fitness costs incurred by reducing the reproductive success of a female relative (see Lehmann & Perrin 2003 for similar arguments on female inbreeding strategies). The number of eggs produced by a female, on the other hand, is largely independent of the number of copulation partners (Bateman 1948; but see Arnqvist & Nilsson 2000), and the reproductive success of a female will depend on the numbers of eggs fertilized by related and unrelated partners: the more eggs fertilized by a relative, the lower is female reproductive success. All else being equal, the reproductive success of a promiscuous female can be simplified as:

$$\left(\frac{u+r}{O}\right)up+\left(\frac{u+r}{O}\right)r(p-\delta),$$

where u and r are the numbers of unrelated and related males, respectively, to inseminate a female, and O is the number of ova produced by the female (i.e. clutch size). Therefore, females are expected to avoid insemination by relatives whenever they are likely to obtain enough sperm for fertilization from unrelated males and when the cost of inbreeding (δ) exceeds inclusive fitness benefits gained through additional reproductive success of a male relative (Lehmann & Perrin 2003). Second, anisogamy often results in a male-biased operational sex ratio (Shuster & Wade 2003), thus reducing the number of potential partners for males relative to females. In species with limited dispersal, this difference may translate into females having several unrelated partners to select from, and for males, relatively few unrelated females available for reproduction. This discrepancy may promote sex-specific dispersal strategies (Perrin & Mazalov 2000). However, when dispersal is constrained the risk of inbreeding may be influenced by sexual selection (Lehmann & Perrin 2003) both before insemination, through the selection of copulation partners and in episodes of sexual selection arising during and following insemination: differential male sperm investment (Wedell *et al.* 2002; Pizzari *et al.* 2003) and female selection of sperm (cryptic female choice, Eberhard 1996; Birkhead

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& Pizzari 2002). These episodes of sexual selection occurring during or after insemination are generally difficult to study, particularly in internally fertilizing species (Stockley 1999; Birkhead & Pizzari 2002), and their role in inbreeding may be particularly confounded by post-zygotic mechanisms. For example, early embryo mortality may reduce the reproductive success of genetically related partners (Birkhead *et al.* 2004). Therefore, the strategies through which each sex influences inbreeding remain poorly understood (Mack *et al.* 2002; Tregenza & Wedell 2002; Bretman *et al.* 2004).

Red junglefowl (*Gallus gallus*) live in small, sexually promiscuous populations where reproductive skew, limited dispersal of both sexes (Collias & Collias 1996) and male sexual coercion (Pizzari *et al.* 2002) create a potential for inbreeding: around 4% of the copulations observed in free-ranging, unconstrained groups of red junglefowl occurred between brothers and sisters, and also between mothers and sons ($n = 135$; Collias & Collias 1996). In addition, inbreeding is known to depress different fitness components in the domestic fowl, *G. gallus domesticus* (e.g. Craig & Baruth 1965; Cheng *et al.* 1985; Abplanalp *et al.* 1992), the domestic subspecies of the red junglefowl (Fumihito *et al.* 1996).

Here, we used experimental techniques to test: (i) female sperm selection based on male relatedness, and (ii) differential male propensity to copulate and invest sperm on the basis of female relatedness. We reveal sex-specific, counteracting responses that influence the risk of inbreeding.

2. MATERIAL AND METHODS

(a) *Study population*

We studied a random-bred population ($n > 60$) of red junglefowl at the Swedish University of Agricultural Sciences, Skara (see Carlborg *et al.* 2003 for further details). We individually labelled eggs from 18 isolated pairs and hatched them in two batches (February and March 2002). Eggs from different pairs were separated by wire partitions within the incubator to assign the maternity and paternity of individual chicks (i.e. pedigree-hatching). Birds were individually marked at hatching and raised on the floor in indoor pens ($3\,\text{m} \times 3\,\text{m}$) in six mixed-sex groups of about 14 individuals, under standardized housing conditions and with food and water *ad libitum*. When females reached sexual maturity (24 October 2002), males were physically (but not visually or acoustically) separated from females. From incubation until the beginning of the experiment (4 February 2003), siblings were randomly distributed across groups within each batch (the two incubation batches were kept separate to avoid confounding effects arising from slight age differences during ontogeny). This ensured that socially familiar (i.e. members of the same group) and unfamiliar birds had the same probability of being full siblings or unrelated, and that a male was sometimes exposed to a familiar female and at other times to an unfamiliar female during experimental trials (see § 2c).

(b) *Insemination trials*

We exposed a male to either his full-sib sister or an unrelated female, allowed him to become familiarized with the female by holding the female facing the male for one minute, following which we held the female in a soliciting position (Pizzari *et al.* 2003) for 20 minutes and allowed him to inseminate her. Male fowl may sometimes mount a female without ejaculating sperm or even without delivering any semen (Pizzari *et al.* 2003). To control for this possibility, we allowed a male to inseminate a female for a maximum of two times within the 20 minutes of a trial. Copulation success was further confirmed by the presence of traces of semen around the female cloaca immediately following a mounting. After a minimum of 72 h, when his sperm reserves were fully replenished (Pizzari *et al.* 2003), a male was allowed to inseminate the other type of female (i.e. either an unrelated female or his sister). To eliminate a potential treatment order effect, the order with which males were presented to a sister and to an unrelated female was alternated in a balanced design. Following a single insemination trial, females were isolated in individual pens and eggs collected for 10 days (Froman *et al.* 2002). Two weeks after insemination we confirmed depletion of sperm stored by females by the absence of sperm on eggs, and exposed the females to a second insemination trial in which a female was exposed to the male type complementing that in the first insemination trial (e.g. if a female was mated with her brother in the first trial, she was exposed to an unrelated male in the second trial).

Freshly laid eggs were opened and the number of sperm that had reached an ovum during the period of time available for fertilization (i.e. following ovulation, when the ovum is in the body cavity and upper infundibulum; Olsen & Neher 1948) was quantified by the number of hydrolysis points on the outer perivitelline layer (PVL) of the egg (galliformes: Wishart 1987; Steele *et al.* 1994; Froman *et al.* 2002; see Birkhead *et al.* 1993 for non-galliformes). In the fowl, the probability that an ovum is fertilized is a function of the number of sperm trapped in the PVL (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 way an ejaculate was stored (Brillard 1993) and remained fertile inside a female (Wishart 1987). In particular, the intercept of the linear regression of the log-transformed number of PVL hydrolysis points in eggs laid over successive days following insemination is proportional to the number of sperm of that insemination that a female retained and stored in the sperm storage tubules (SSTs) (Froman *et al.* 2002). We tested the idea that females reduce the risk of inbreeding in two ways. First, we analysed variance in the numbers of PVL hydrolysis points in eggs laid over successive days following incestuous and unrelated inseminations within each female, using SAS (Der & Everitt 2001), through a generalized linear mixed model with restricted maximum-likelihood estimation (REML GLMM), Poisson error distribution and log link function, stepwise deletion of non-significant terms, number of PVL perforations as the dependent variable, partner relatedness, familiarity (i.e. whether a male and a female had been raised together) and number of copulations (one or two) during a trial (entering number of copulations as a covariate did not change the results) as fixed effects, female and male identity as random factors and oviposition day from 1 to 10 as covariate. Second, we generated linear regressions of log-transformed PVL hydrolysis point numbers over time for each insemination trial in which a female produced three or more eggs following insemination, and compared intercepts of the regressions obtained from trials with related and unrelated males within individual females, through a Wilcoxon paired test, using SPSS software.

(c) *Allocation trials*

We quantified male sperm investment in a sister and an unrelated female by using males in another set of similar trials in which females were fitted with a harness covering the cloaca. This enabled us to collect natural ejaculates and measure the volume of semen ejaculated to the nearest 0.5 μl with a pipette (Pizzari *et al.* 2003). To avoid a potential trial order effect we exposed half of the males to a sperm allocation trial first and subsequently to an insemination trial with the same females, and the other half to the insemination trial first followed 3 days later by the allocation trial. We measured male propensity to copulate as the time (to the nearest 0.5 minute) it took a male to mount a female immediately after she was presented to him. Related partners in the experiment were not significantly more likely to be familiar (i.e. raised together) than unrelated partners (insemination trials $\chi^2_1 = 0.29$, $p = 0.59$, n pairs = 29; allocation trials $\chi^2_1 = 0.30$, $p = 0.58$, n pairs = 28). Therefore, males could not use familiarity cues to assess partner relatedness. In addition, males may recognize females on the basis of the size of the female comb and/or female body mass (e.g. Pizzari *et al.* 2003). Each female was weighed to the nearest 0.1 g and female comb height and length measured to the nearest 0.01 mm with a digital calliper. Related and unrelated females presented to a male were matched to minimize differences in these traits. There was no significant difference in comb length (insemination trials: Wilcoxon paired test, $Z = -0.06$, $p = 0.95$; allocation trials: $Z = -0.06$, $p = 0.95$, n males = 28), comb height ($Z = -0.22$, $p = 0.83$; $Z = -0.19$, $p = 0.85$) and body mass ($Z = -0.06$, $p = 0.95$; $Z = -0.01$, $p = 0.99$) of related and unrelated females presented to individual males. We tested the idea that males respond differentially to related and unrelated females in three ways. First, we analysed variance in:

(i) time elapsed to first mounting averaged over insemination and allocation trials, and
(ii) total number of sperm produced when a male copulated with a related female (i.e. first + second copulation) and when he copulated with an unrelated female, through Wilcoxon paired tests, using SPSS software.

We considered trials in which a male did not mount a female as maximal time to copulation (i.e. 20 min) and analysed the propensity to copulate with and without such trials for the 28 males.

Second, we further investigated the effect of female relatedness on male response, controlling for partner familiarity, using SAS, through:

(i) REML GLMM with Poisson error distribution and log link function, stepwise deletion of non-significant terms, time to first copulation as the dependent variable, familiarity and relatedness of partner as fixed factors and male and female identity as random factors, and
(ii) REML GLMM with Gamma error distribution, stepwise deletion of non-significant terms, number of sperm contained in an ejaculate as the dependent variable, copulation order (first and second with a female), familiarity and relatedness of partner as fixed factors, and male and female identity as random factors.

Finally, we tested the idea that males respond differentially to related and unrelated partners via seminal fluid, because some seminal fluid products mediate the fertilizing performance of an ejaculate in some species (Chapman 2001). We tested this idea using SAS through REML GLMM with Gamma error distribution, stepwise deletion of non-significant terms, volume of semen released in each copulation with a female as the dependent variable, copulation order (first and second with a female), partner relatedness and familiarity as fixed factors, male and female identity as random factors and sperm number as a covariate.

We used 28 males and 28 females for the insemination trials. We excluded four trials (one female, two males) in which one of the females laid fewer than three eggs following insemination. The same 28 males were exposed to the same 28 females in the allocation trials with the exception of one male that was exposed to the full-sib sister of the female he inseminated in the insemination trial. We excluded one male that failed to inseminate either female.

3. RESULTS

(a) *Female response*

Female fowl have limited control over copulation, particularly when other males are not present to interrupt (Pizzari *et al.* 2002). However, female fowl can influence paternity after insemination (Pizzari *et al.* 2002; Birkhead *et al.* 2004). Therefore, we tested the idea that female fowl reduce the risk of incestuous fertilization after insemination by selecting against the sperm of their brothers. Consistent with this idea, females retained fewer sperm following insemination by brothers than by unrelated males, thus reducing the probability of an incestuous insemination resulting in fertilization (Wishart 1997). First, controlling for the effect of oviposition order, the number of sperm reaching the eggs of a female was significantly higher following insemination by an unrelated male than by her brother (figure 1). This effect was entirely dependent on male genetic relatedness and not social familiarity (figure 1 legend). Second, the number of sperm initially stored in the female sperm storage organs was significantly higher when a female was inseminated by an unrelated male than when she was inseminated by a brother (figure 1 legend). This result can be explained by two non-mutually exclusive mechanisms:

(i) post-copulatory female discrimination against the sperm of related males, and
(ii) males investing more sperm in unrelated females.

To disentangle these mechanisms we quantified male responses to sisters and unrelated females.

(b) *Male response*

Consistent with the idea that in the absence of unrelated partners males invest in rather than avoid inbreeding, in the allocation trials males had a similarly high probability of copulating with related and unrelated females (Wilcoxon paired test, $Z = -1.414$, $p = 0.157$, $n = 27$): out of 27 males, 25 (93%) copulated with both related and unrelated females, whereas two did not copulate with the unrelated female. Consistent with these results, all females laid at least one egg containing some sperm following the insemination trials, indicating that males copulated and inseminated sperm in both types of females during these trials also. In addition, there was a weak tendency for males to initiate copulation faster when presented with an unrelated female than when presented with a sister (figure 2), suggesting that males may recognize female relatedness and may be more hesitant to copulate with a relative.

Importantly, consistent with the idea that females discriminate cryptically against incestuous insemination, males did not inseminate more sperm in unrelated females (figure 3). In fact, contrary to the idea that males invest more sperm in unrelated partners, there was a tendency for males to inseminate more sperm into sisters than into unrelated females (figure 3). Similarly, variance in the volume of semen ejaculated by a male into a female was explained by copulation order and the number of sperm contained in

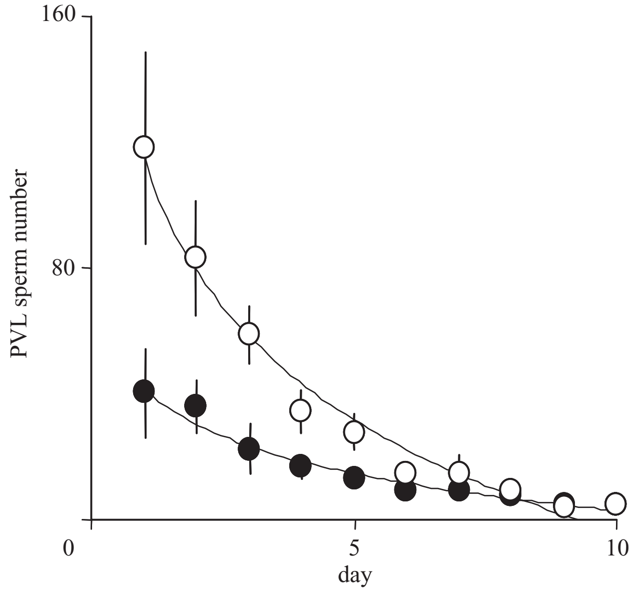

Figure 1. Female response to inbreeding. Females retained more sperm following insemination by an unrelated male. More sperm reached the perivitelline layer (PVL) of eggs produced by a female for at least the first five days following insemination by an unrelated male than insemination by a brother (GLMM, number of copulations: $F_{1,303} = 3.25$, $p = 0.072$, day: $F_{1,303} = 163.17$, $p < 0.0001$, relatedness: $F_{1,303} = 43.04$, $p < 0.0001$, familiarity: $F_{1,303} = 0.64$, $p = 0.42$, day × relatedness: $F_{1,303} = 3.01$, $p = 0.084$). Open data points: unrelated inseminations, logarithmic regression curve: PVL count $= -51.65 \pm 2.58 \ln(\text{day}) + 114.89 \pm 4.28$, adj. $r^2 = 0.98 \pm 5.67$, $p < 0.0001$; closed data points: incestuous inseminations, logarithmic regression curve: PVL count $= -17.01 \pm 1.10 \ln(\text{day}) + 42.01 \pm 1.84$, adj. $r^2 = 0.96 \pm 2.43$, $p < 0.0001$; error bars: ±1 s.e.m. More sperm were initially released from a female's SSTs following unrelated insemination than insemination by her brother, as indicated by the within-female comparison of intercepts of linear regressions of log-transformed number of PVL hydrolysis points over time (Wicoxon paired test intercepts, $Z = -3.83$, $p < 0.0001$, Sign test, $p < 0.0001$, n females $= 24$).

the ejaculate and not by either female familiarity or relatedness (GLMM, sperm number: $F_{1,92} = 46.55$, $p < 0.0001$; copulation order: $F_{1,99} = 6.11$, $p = 0.015$; relatedness: $F_{1,98} = 0.43$, $p = 0.51$; familiarity: $F_{1,99} = 0.01$, $p = 0.9167$; relatedness × familiarity: $F_{1,98} = 1.51$, $p = 0.22$).

4. DISCUSSION

Together, the results of the present study indicate that, consistent with theory, whereas female fowl selected against the sperm of related partners after insemination, male fowl did not avoid inbreeding and did not invest less in copulations with related females when these were the only females available. In fact, our results suggest that males may recognize kin and invest more sperm in copulations with familiar, related females when unrelated females are not available, thus ameliorating cryptic female choice against inbreeding.

(a) *Female sperm selection*

Our results provide experimental evidence that despite receiving on average more sperm from brothers, female fowl were able to reduce the probability that the sperm of a genetically related partner will fertilize an egg, through post-insemination selection against the sperm of related (but not necessarily socially familiar) partners. The genetic similarity of reproductive partners may influence variation in paternity in some species (Tregenza & Wedell 2000; Kraaijeveld-Smit *et al.* 2002; Garner & Schmidt 2003; Olsson *et al.* 2003; Stockley 2003). In *Drosophila melanogaster* (Mack *et al.* 2002) and the field cricket *Gryllus bimaculatus* (Tregenza & Wedell 2002; Bretman *et al.* 2004), ejaculates from males related to the inseminated female are disadvantaged in competition with the ejaculates of unrelated males and, all else being equal, fertilize fewer eggs than unrelated inseminations. Pre-zygotic male and female mechanisms occurring during and after an insemination (i.e. male sperm investment and cryptic female choice) and post-zygotic constraints (i.e. inbred embryos suffering higher mortality) may contribute to explaining such biases in paternity (Birkhead *et al.* 2004). A similar effect, independent of male sperm investment and differential embryo mortality, has been observed in the field cricket *Grylloides supplicans* (Stockley 1999). Our results are consistent with those of these studies and demonstrate that cryptic female choice reduces the risk of inbreeding and favours fertilization by genetically more compatible partners, independently of male differential sperm allocation, differential embryo mortality and partner social familiarity. Male relatedness to a partner appears to determine the proportion of sperm stored by a female fowl in the SSTs. Because the relative number of sperm from multiple males reaching an ovum is a powerful determinant of the outcome of sperm competition (Martin *et al.* 1974) and because female fowl typically

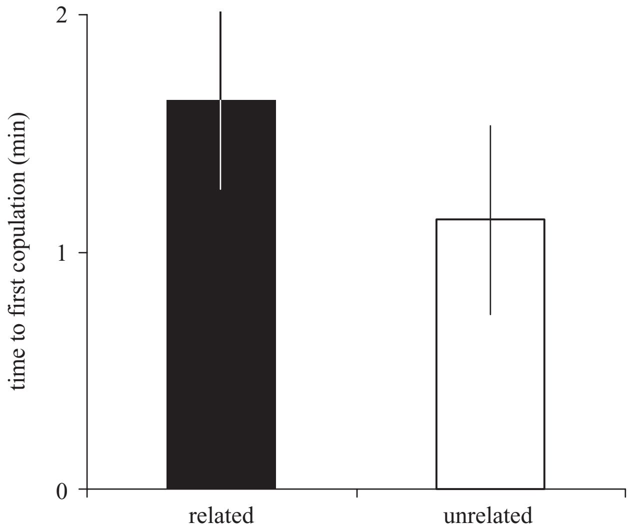

Figure 2. Male behavioural response to inbreeding. Mean (± s.e.m.) time to first copulation was significantly shorter when a male was exposed to an unrelated than to a related female (Wicoxon paired test of time to first copulation, $Z = -2.32$, $p < 0.021$, Sign test $p = 0.003$, n males $= 28$). This trend was even stronger when trials in which males did not copulate with a female were excluded ($Z = -2.66$, $p = 0.008$, Sign test $p = 0.003$, n males $= 28$). However, this tendency disappeared when variance in time to first copulation was partitioned between partner familiarity and relatedness (GLMM, relatedness: $F_{2,115} = 0.00$, $p = 0.97$, familiarity: $F_{1,116} = 1.08$, $p = 0.30$, relatedness × familiarity: $F_{1,115} = 0.06$, $p = 0.80$).

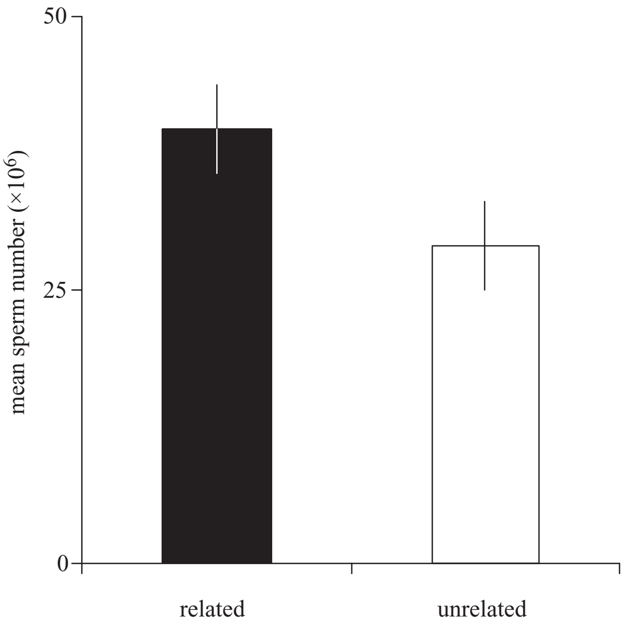

Figure 3. Inbreeding and male sperm investment. Mean (± s.e.m.) total sperm investment (first plus second copulation) was significantly higher when a male inseminated his sister versus an unrelated female (Wilcoxon paired test of relative sperm investment, $Z = -2.07$, $p = 0.039$, Sign test, $p = 0.052$, n males = 27). The trend for males to invest more sperm in sisters remained after excluding two males that failed to mount a female during a trial and two males that copulated but did not deliver sperm to one of the females ($Z = -1.98$, $p = 0.048$, Sign test, $p = 0.093$, n males = 23). However, this effect appeared to be mostly mediated by partner familiarity (GLMM, relatedness: $F_{1,101} = 2.07$, $p = 0.15$, familiarity: $F_{1,101} = 5.35$, $p = 0.022$, relatedness × familiarity: $F_{1,101} = 0.11$, $p = 0.74$, copulation order: $F_{1,101} = 21.02$, $p < 0.0001$).

copulate with multiple males within a reproductive event (the production of a clutch of eggs; Pizzari *et al.* 2002), female choice against related sperm will bias sperm competition in favour of the ejaculates of unrelated males. It is interesting to note that female selection against sperm of related partners does not translate into a risk of producing infertile eggs. The probability that an egg is fertilized is less than 1 for fewer than 2 hydrolysis points per mm^2 on the outer PVL around the blastodisc (Wishart 1997). Neither incestuous nor unrelated inseminations generated densities below this threshold, as they both approached 5 hydrolysis points per mm^2 on the last oviposition day (day 10). This may be a result of: (i) males inseminating more sperm into related females, and (ii) females biasing the outcome of sperm competition in favour of unrelated males but simultaneously minimizing the risk of egg infertility, by allowing a minimal number of sperm to reach the ova. In other words, females may trade off the risk of unfavourable fertilizations against the risk of infertility (Ball & Parker 2003). The mechanisms mediating female preference for the sperm of unrelated partners are unclear. One possibility is that cryptic female choice against inbreeding is mediated by complementarity at major histocompatibility complex (MHC) haplotypes (Tregenza & Wedell 2000; Olsson *et al.* 2003). These haplotypes are expressed on the sperm surface in some species (Martin-Villa *et al.* 1999; Ziegler *et al.* 2002) and, following insemination, on the oviduct of domestic fowl (Zheng *et al.* 2001). Post-insemination female selection against the sperm of genetically related and incompatible males may be a powerful force driving the evolution of female polyandry (Zeh & Zeh 1997; Tregenza & Wedell 2000). Female fowl are subjected to intense sexual harassment and have evolved subtle mechanisms to influence paternity, including differential sperm ejection, an example of directional cryptic female choice favouring the dominant male phenotype (Pizzari *et al.* 2002). Female fowl may also select sperm of different males in a non-directional way, based on the genetic compatibility of partners (Birkhead *et al.* 2004). Our study provides an experimental demonstration of this idea and the results suggest that directional sexual selection for male social status in this species (Pizzari *et al.* 2002) may be buffered by non-directional female strategies of inbreeding avoidance.

(b) *Male response to inbreeding*

When exposed to only one female, male fowl were equally likely to copulate with related and unrelated females. In addition, the present study provides some evidence that male fowl may be able to recognize females that are likely to be genetically related, possibly through social familiarity cues. The observation that only *ca.* 4% of copulations occurred between first-order relatives in free-ranging, unconstrained groups of red junglefowl, despite the limited dispersal of these birds (Collias & Collias 1996), is consistent with the idea that males may hesitate to copulate with relatives to obtain cues on the current availability of unrelated females (females may possibly do the same, although we did not test this). However, when unrelated females are unavailable, it may pay males to inseminate relatives and compensate for the disfavoured role played by their ejaculates in incestuous copulations by inseminating more sperm. It is therefore possible that male strategies to achieve incestuous fertilization may counteract female efforts to select against the sperm of related males, thus helping to explain the lack of female sperm discrimination found in some studies (Stockley 1997). Our results suggest that the male differential response to related and unrelated partners may be mediated by familiarity cues. Experimental evidence suggests that in some galliformes, interactions between reproducing individuals may be mediated by both kin recognition through associative learning (Bateson 1982) and unlearnt kin recognition, possibly through self-referent phenotype matching (Bateson 1982; Petrie *et al.* 1999). The effects of both relatedness and familiarity detected in our study are weak. This may be a result of high between-male variance in male response to related and unrelated females. For example, differences in access to females determined by male status may generate different strategies of copulation behaviour and sperm investment (e.g. Pizzari *et al.* 2003), thus constraining the potential of our study to test kin recognition by male fowl and determine the role of social familiarity and relatedness. The mechanisms of kin recognition mediating male partner choice in birds remain unclear and deserve further research.

In conclusion, the present study reveals that constraints on dispersal may set the scene for the evolution of mechanisms of kin recognition (behavioural in males, possibly mediated by social familiarity; physiological in females, possibly mediated by sperm:oviduct interactions) mediating sex-specific responses and counter-responses driven by inter-sexual conflict over inbreeding. More generally, these results indicate that different male- and female-specific mechanisms interact to determine paternity and thus caution should be used when inferring evolutionary mechanisms from patterns of variation in paternity derived from natural copulations.

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.