

Inbreeding avoidance in spiders: evidence for rescue effect in fecundity of female spiders with outbreeding opportunity

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Abstract

Selection by inbreeding depression should favour mating biases that reduce the risk of fertilization by related mates. However, equivocal evidence for inbreeding avoidance questions the strength of inbreeding depression as a selective force in the evolution of mating biases. Lack of inbreeding avoidance can be because of low risk of inbreeding, variation in tolerance to inbreeding or high costs of outbreeding. We examined the relationship between inbreeding depression and inbreeding avoidance adaptations under two levels of inbreeding in the spider *Oedothorax apicatus*, asking whether preference for unrelated sperm via pre- and/or post-copulatory mechanisms could restore female fitness when inbreeding depression increases. Using inbred isofemale lines we provided female spiders with one or two male spiders of different relatedness in five combinations: one male sib; one male nonsib; two male sibs; two male nonsibs; one male sib and one male nonsib. We assessed the effect of mating treatment on fecundity and hatching success of eggs after one and three generations of inbreeding. Inbreeding depression in F1 was not sufficient to detect inbreeding avoidance. In F3, inbreeding depression caused a major decline in fecundity and hatching rates of eggs. This effect was mitigated by complete recovery in fecundity in the sib–nonsib treatment, whereas no rescue effect was detected in the hatching success of eggs. The rescue effect is best explained by post-mating discrimination against kin via differential allocation of resources. The natural history of *O. apicatus* suggests that the costs of outbreeding may be low which combined with high costs of inbreeding should select for avoidance mechanisms. Direct benefits of post-mating inbreeding avoidance and possibly low costs of female multiple mating can favour polyandry as an inbreeding avoidance mechanism.

Introduction

Incestuous matings are predicted to depress fitness through the expression of homozygous deleterious recessive alleles or the loss of heterozygote advantage (Charlesworth & Charlesworth, 1987; Roff, 2002). The cost of inbreeding is expected to shape mating strategies and drive the evolution of mating biases favouring nonconsanguineous mates (Blouin & Blouin, 1988; Pusey & Wolf, 1996). Hence, the prevailing framework

for understanding the evolution of mating preferences relies on inbreeding depression as a major selective force (Crnokrak & Roff, 1999; Amos *et al.*, 2001; Keller & Waller, 2002).

Inbreeding avoidance mechanisms have received much attention in a broad range of animal taxa and fall into two broad categories: precopulatory and post-copulatory avoidance. Precopulatory mechanisms include dispersal of one or both sexes (Pusey, 1987; Johnson & Gaines, 1990); protandry (Morbey & Ydenberg, 2001), delayed maturation (Dewsbury, 1988) or reproductive suppression (Wolff, 1992). In direct encounters, precopulatory incest avoidance may occur through kin recognition (Fletcher & Michener, 1987; Penn & Potts, 1999) or via preference for genetically

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dissimilar mates (Tregenza & Wedell, 2000). For example, the major histocompatibility complex (MHC) in vertebrates that plays a critical role in immune reactions can be used as a genetic matching system to detect dissimilarity with respect to self. MHC-dependent mating preferences may therefore function to avoid inbreeding (Penn & Potts, 1999).

Polyandry, or female multiple mating, sets up post-copulatory opportunities for gamete selection to operate (Stockley *et al.*, 1993; Newcomer *et al.*, 1999). The risk of inbreeding may be reduced via cryptic processes of sperm selection or sperm competition which result in paternity bias towards unrelated or genetically compatible sires (Eberhard, 1996; Zeh & Zeh, 1996; Birkhead & Møller, 1998; Tregenza & Wedell, 2000). The adaptive significance of post-mating inbreeding avoidance relies on the coevolution of polyandry and mechanisms of paternity bias in favour of unrelated mates (Mack *et al.*, 2002; Tregenza & Wedell, 2002; Bretman *et al.*, 2004).

A large body of empirical evidence collectively suggests that inbreeding avoidance has shaped the evolution of mating systems. Nevertheless, a number of studies have failed to detect preference for unrelated mates, whether via precopulatory mate choice (Keller & Arcese, 1998; Keller & Fournier, 2002; Haikola *et al.*, 2004; Bilde *et al.*, 2005) or post-copulatory mechanisms (Stockley, 1997, 1999; Cunningham & Cheng, 1999; Jennions *et al.*, 2004). The ambiguity in the empirical evidence could stem from differential selection pressures experienced in wild populations (Barrett & Charlesworth, 1991; Crnokrak & Barrett, 2002; Jennions *et al.*, 2004). Relaxed selection on inbreeding avoidance adaptations may apply in at least two situations: in populations with a low risk of experiencing inbreeding, for example, when mobility and effective population size is high, inbreeding avoidance mechanisms could be selected against if these are costly to maintain. Secondly, relaxed selection on inbreeding avoidance may occur in populations where inbreeding depression is mild. For example, Duarte *et al.* (2003) found no behavioural inbreeding avoidance in white-toothed shrew (*Crocidura russula*), but also the absence of phenotypic inbreeding depression; and a study of spiders with a history of inbreeding, showed mild inbreeding depression and no avoidance of sib matings (Bilde *et al.*, 2005).

Here we aim to explore the relationship between inbreeding and inbreeding avoidance adaptations; specifically, we investigate whether there is tolerance to inbreeding under varying degree inbreeding, and ask whether preference for unrelated sperm via precopulatory and/or post-copulatory mechanisms can restore female fitness when inbreeding depression increases. To answer these questions, we conducted experimental studies of inbreeding avoidance in a linyphiid spider *Oedothorax apicatus*. In this species, high effective population size and high mobility (Thornhill, 1983; Alderweireldt, 1987, 1989, 1994; Bell *et al.*, 2005), may

result in a low realized risk of inbreeding which may contribute to relaxed selection for inbreeding avoidance.

We created inbred isofemale lines and tested for inbreeding depression and evidence for inbreeding avoidance after one and three generations of full-sib matings. Female spiders were presented with either one or two male spiders of different relatedness in five treatment combinations: one male sib; one male nonsib; two male sibs, two male nonsibs and finally one male sib + one male nonsib, and the effect of mating treatment on fecundity and hatching success of eggs was examined. Under inbreeding avoidance, we predicted that female spiders would recover fitness through paternity bias in favour of unrelated male spiders, or through differential investment in offspring from outbred crosses. Our results support the latter hypothesis. After three generations of inbreeding, we found severe inbreeding depression in fecundity and hatching rate of eggs. Outbreeding opportunities provided a recovery in fecundity whereas an intermediate hatching rate suggested random sperm mixing.

Materials and methods

Study species

Mated female spiders *O. apicatus* Blackwell (Araneae, Linyphiidae) were collected in fallow fields in Eastern Jutland, Denmark, in April 2004. The female spiders were used as individual founders of inbred female isolines ($n = 54$ founders). Offspring of these female spiders were raised individually until maturity. For each female isolate, one pair of full siblings were mated to create the first generation (F1) of inbred offspring. From each isolate a set of the F1 offspring were used in the first experiment (see below). F2 and F3 inbred generations were created by within-line matings among full-sib pairs.

All spiders were kept at 20°C and a 16 : 8 h light : dark cycle, in cylindrical plastic tubes 7 × 1.5 cm with a 2-cm bottom layer of moist plaster-of-Paris mixed with charcoal. Tubes were sealed with a foam rubber lid. Juvenile spiders were fed live collembolans (*Sinella curviseta* and *Tomocerus bidentata*), and adults were fed fruit flies (*Drosophila melanogaster*). Food was provided *ad libitum* once a week.

F1: inbreeding depression and inbreeding avoidance

Virgin F1 female spiders were assigned to five treatment groups according to relatedness of their mates: (1) sib ($n = 41$); (2) sib-sib ($n = 24$); (3) sib-nonsib ($n = 20$); (4) nonsib ($n = 33$); (5) nonsib-nonsib ($n = 22$). With this design, we wanted to: (1) determine the effect of full-sib inbreeding when mated with a single mate; (2) test the effect of one vs. two male spiders on female reproductive success; (3) given there was inbreeding depression, to determine whether inbreeding avoidance would provide a rescue effect in the sib-nonsib group.

Unrelated male spiders were from a different inbred isolate than the subject female spider. In this way, potential effect of inbreeding on reproductive performance was controlled across all treatment groups. One to two female spiders from each isolate were used in each treatment group. Mating was not directly observed; rather the male spiders were confined with the female spider until an egg sac was produced, after which all spiders were removed. Egg sacs were checked daily for hatching. Upon hatching, the offspring were counted and the egg case was opened to determine clutch size and hatching rate.

F3: inbreeding depression and inbreeding avoidance

We repeated the experiment described above using adult virgin F3 male and female spiders from each inbred isolate. Each female spider from the inbred lines was assigned to mating experiments using the same procedure as in the F1 experiment (sib, $n = 67$) (sib-sib, $n = 23$) (sib-nonsib, $n = 18$) (nonsib, $n = 70$) (nonsib-nonsib, $n = 19$).

Data on proportions (production of egg sac and egg hatching success) were analysed using Generalized Linear Models using quasibinomial errors (GLM-qb) as the

data showed overdispersion because of unexplained variance. Differences between treatment levels were identified *post hoc* with GLM contrasts using *t*-statistics on parameters (Crawley, 2002). GLM were performed within R (R Development Core Team, 2004).

Results and discussion

After one generation of inbreeding, the number of eggs laid in the first egg sac differed significantly between treatment groups (*anova*, $F_{4,97} = 5.49$, $P = 0.0005$), however, *post hoc* comparisons showed that a higher fecundity in the nonsib-nonsib group compared with the remaining groups accounted for most of the observed difference (Fig. 1). Hatching success of eggs did not vary significantly across mating treatments in F1 (GLM-qb, $\chi^2_4 = 32.89$, $P = 0.16$; Fig. 1). It appears that inbreeding depression was not sufficiently severe to be detected after one generation of sib-mating and therefore also not the potential rescue effect via outbreeding. We note that by using wild-caught female spiders that could have mated multiply prior to oviposition, the sons and daughters that were used to create F1 inbred offspring may have been half-sibs instead of full sibs. This implies that significant effects of inbreeding are conservative, as relatedness

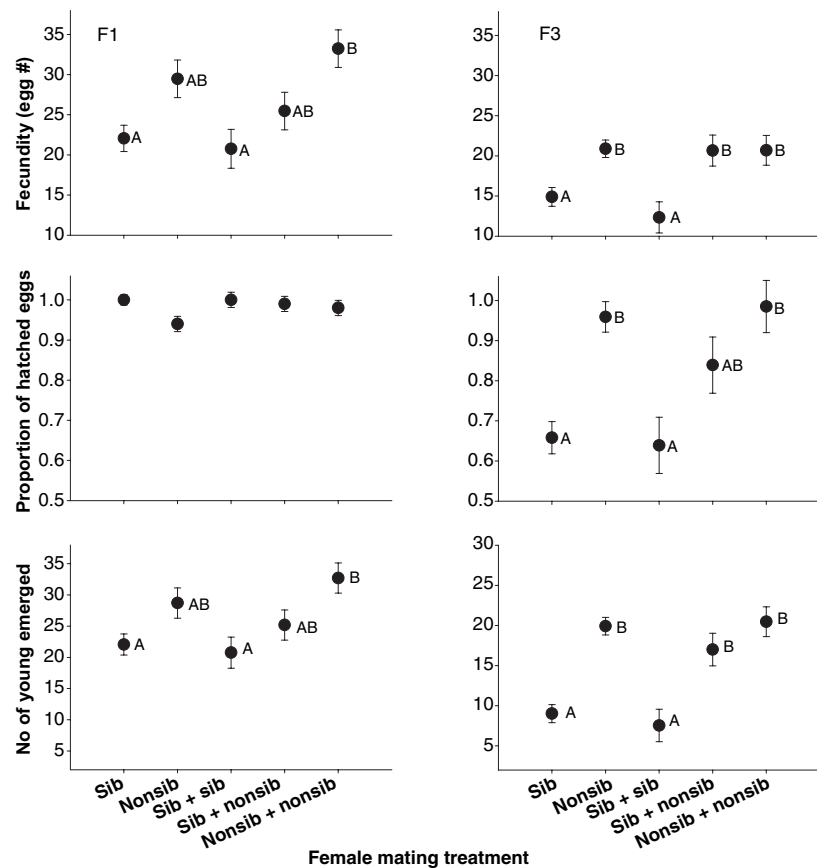


Fig. 1 Data on reproductive performance of first (F1) and third (F3) inbred generation of female spiders in five treatment groups according to the number and relatedness of male spiders (one male sib; one male nonsib; two male sibs; one male sib and one male nonsib; two male nonsibs). Data are mean \pm SE. Values not connected by the same letter are significantly different using *post hoc* contrast analysis of inbreeding (sib + sib-sib) vs. outbreeding (nonsib + nonsib-nonsib); inbreeding vs. choice for outbreeding (sib-nonsib) and outbreeding vs. choice for outbreeding.

could have been lower than expected in inbred matings and inbreeding depression therefore weaker than under full-sib matings in F1.

In contrast, after three generations of inbreeding, there was a strong effect on fecundity revealed by a decline in egg number of approximately 35% in the inbred (sib and sib-sib) treatments ($F_{4,103} = 6.61$, $P < 0.0001$, Fig. 1). We compared the three two-male groups (*sensu* Tregenza & Wedell, 2002) and found that fecundity was completely restored in the sib-nonsib group where inbreeding avoidance could operate (within-model contrasts: sib-nonsib vs. sib-sib: $t_{34} = 3.03$, $P = 0.003$; nonsib-sib vs. nonsib-nonsib: $t_{34} = -0.01$, $P = 0.99$). Under a model assuming equal contribution from both sires the expected fecundity would be significantly lower than the observed fecundity in the sib-nonsib group ($t_{24} = -2.31$, $P = 0.029$). Hence, when provided with outbreeding opportunities in the sib-nonsib group fecundity was significantly improved compared with the additive scenario of random contribution from both male spiders, explicitly supporting the rescue hypothesis.

The effect of mating treatment on hatching rate was also highly significant in F3 (GLM-qb, $\chi^2_4 = 365.6$, $P < 0.0001$; Fig. 1) because of a reduced hatching rate in the sib and sib-sib groups (contrasts, *t*-test, $t_{105} = 5.39$, $P < 0.0001$). Comparing the three two-male groups revealed that hatching success was significantly higher in the sib-nonsib group than the sib-sib group (*post hoc t*-test, $t_{21} = 2.92$, $P = 0.004$) but lower than in the nonsib-nonsib group (*post hoc t*-test, $t_{22} = 2.17$, $P = 0.03$). This means that female spiders in the sib-nonsib group had an egg hatching success halfway between completely outbreeding and completely inbreeding female spiders. The explicit test of this was done by comparing the mean hatching success of female nonsib-nonsib and sib-sib vs. that of mated female sib-nonsib ($t_{34} = 0.06$, $P = 0.47$) and suggests that male nonsibs and male sibs contributed an equal amount of sperm that was then used randomly by the female spider. Hatching rate in the sib-nonsib group, therefore, was not improved more than expected from random mixing of sperm from both male spiders.

In contrast to the rescue effect in fecundity found here, previous studies of post-mating inbreeding avoidance have demonstrated a rescue effect in hatching success. Such rescue effect may result from a negative correlation between relatedness and sperm competitive ability (Mack *et al.*, 2002), cryptic differential fertilization in favour of unrelated sperm (Olsson *et al.*, 1996; Tregenza & Wedell, 2002; Bretman *et al.*, 2004, see also Bussiere *et al.*, 2006) or interactions between embryos (Zeh & Zeh, 2006). These effects may be widespread but not universal (Jennions *et al.*, 2004). Although hatching success is commonly used in this field to identify the rescue effect of polyandry in relation to inbreeding depression, there is no strong *a priori* genetic argument for this practice, as inbreeding depression may manifest itself in a number of different ways (Roff, 1997; Keller & Waller, 2002; Zeh & Zeh, 2006).

The rescue effect in fecundity in the sib-nonsib group suggests that processes other than sperm selection may be involved in mitigating the costs of inbreeding (Eberhard, 1996; Pizzari *et al.*, 2004; Zeh & Zeh, 1997, 2006). For example, female spiders could allocate more resources to the production of offspring fathered by unrelated male spiders (Sheldon, 2000); or outbred zygotes might benefit their inbred half-sibs in mixed-paternity broods (Zeh & Zeh, 2006). The actual mechanism involved requires further investigations. In spiders, it has been suggested that female spiders allocate resources quantitatively by altering egg number in response to external factors (Marshall & Gittelman, 1994). However, fine scaled within-species studies also reveal complex relationships between female condition/size and egg size (Tanaka, 1995; Gonzaga & Vasconcellos-Neto, 2001) suggesting plasticity in resource allocation by female spiders. Yolk accumulation occurs in two steps, with the first and small accumulation taking place in young egg cells, whereas the second and much larger accumulation of yolk occurs after copulation (Foelix, 1996), providing an opportunity for female spiders to vary resource allocation in response to male quality.

Inbreeding avoidance and subsequent rescue effect could arise either by behavioural avoidance of incestuous matings (Pusey & Wolf, 1996) or by means of cryptic post-mating processes (Tregenza & Wedell, 2002). Precopulatory preference for unrelated mates requires a recognition system where the phenotypic trait on which choice is based is associated with the genotypic trait that confers genetic benefits to the offspring (Brown & Eklund, 1994; Penn & Potts, 1999). In arthropods, effective recognition systems are often based on cuticle hydrocarbon profiles (Singer, 1998); however, no evidence for inbreeding avoidance based on this recognition system is known (Keller & Fournier, 2002). Although not measured directly, we did not find any evidence for avoidance of sib matings in our study. Firstly, in the no-choice situation, a similar number of female spiders reproduced in the inbred groups compared with the outbred treatments. Secondly, evidence for sperm mixing in the sib-nonsib group suggests that female spiders did not discriminate male sibs. We note, however, that precopulatory avoidance could be conditional, depending on whether there is a choice situation or not. Nevertheless, precopulatory discrimination of kin as mates seems to be uncommon in arthropods (Keller & Fournier, 2002; Tregenza & Wedell, 2002; Haikola *et al.*, 2004; Bilde *et al.*, 2005; Edvardsson, 2005; but see Simmons, 1989, 1991). We therefore consider it more likely that cryptic post-copulatory processes restored fitness in the sib-nonsib group.

Theory predicts inbreeding avoidance to be driven by the balance between inbreeding depression and the costs of outbreeding (Bengtsson, 1978; Parker, 1979; Waser *et al.*, 1986). In this study, the cost of inbreeding increased with the level of inbreeding, and therefore

selection for avoidance adaptations could be expected. It is possible that the costs of outbreeding are low, because both effective population size and mobility are likely to be high in natural populations of *O. apicatus* (Thornhill, 1983; Alderweireldt, 1987, 1989, 1994; Bell *et al.*, 2005) increasing the likelihood of encountering nonconsanguineous mates. We found that inbreeding avoidance most likely occurred by post-mating processes suggesting adaptive advantages for polyandrous female spiders. We did not detect any effects of male number on oviposition rate (GLM-qb, $\chi^2_1 = 0.16$, $P = 0.77$); fecundity (*anova*, $F_{1,207} = 0.62$, $P = 0.43$) or hatching rate (GLM-qb, $\chi^2_1 = 14.8$, $P = 0.22$), so low costs of female multiple mating and direct benefits of post-mating inbreeding avoidance could favour polyandry in this system.

An important factor in understanding the observed polymorphisms in the role of inbreeding avoidance in mating systems across organisms may be the realized risk of mating with close relatives (Pusey & Wolf, 1996; Jennions *et al.*, 2004). To infer this information it is necessary to acquire accurate information on genetic variability or the frequencies of deleterious recessive alleles in natural populations. Such information may provide insight into the selective forces underlying mating biases such as kin discrimination and be suggestive of the likelihood of such mating biases to persist.

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