

Females benefit from mating with different males in the scorpionfly *Panorpa cognata*

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The adaptive significance of female polyandry has become a recurrent subject of recent theoretical and empirical research. It has been argued that in addition to direct benefits, such as nuptial gifts or an adequate sperm supply, females may gain genetic benefits from mating with different males. Females of the scorpionfly *Panorpa cognata* mate with several males during their lifetime. In an experiment designed to rule out any direct nutritional benefit of multiple matings, I found that polyandrous females that mated with two different males achieved a significantly higher egg-hatching success than monandrous females that mated twice with the same male. However, individual males did not trigger the same response in different females as the egg-hatching success of different females that mated with one and same male did not correlate. The results, thus, do not conform to predictions from hypotheses assuming that genetic benefits of polyandry are influenced by the intrinsic genetic quality of males. The results are, however, consistent with the genetic incompatibility hypothesis. Nevertheless, substances from different males transferred during copulation may synergistically affect zygote viability. Furthermore, I discuss why paternity studies can only explicitly test the genetic incompatibility hypothesis if there are a priori expectations of female-male genome compatibilities. *Key words:* egg-hatching success, genetic incompatibility, Mecoptera, polyandry, sperm limitation. [*Behav Ecol* 17:435–440 (2006)]

Due to the advancement and application of molecular genetic techniques, it has increasingly been acknowledged that females of many animal species mate with several males (see Birkhead and Møller, 1998; Eberhard, 1996; Sauer and Lubjuhn, 1999). The traditional view has been that female fitness should in general not increase with matings additional to the one that is necessary for fertilization (Bateman, 1948). Considering the potentially high risks and costs involved (Chapman et al., 2003; Rowe et al., 1994), the frequent occurrence of polyandry constitutes a problem for evolutionary biologists as to why selection should favor its evolution (for review see, e.g., Arnqvist and Nilsson, 2000; Jennions and Petrie, 2000).

There are two categories of hypotheses dealing with the potential benefits of polyandry. First, by mating with multiple males, females may derive direct benefits that increase their survivorship, fecundity, or parental investment. Females can benefit, for instance, by receiving nutritional nuptial gifts, seminal fluids that stimulate female reproductive physiology and behavior, a sufficient sperm reserve, or additional paternal care from their mating partners (Arnqvist and Nilsson, 2000).

Second, several hypotheses on how females may gain genetic benefits from polyandry have been proposed (see Jennions and Petrie, 2000). It has been argued that polyandry may be beneficial as it increases the genetic diversity of the offspring. For instance, if environmental conditions are unpredictable, increasing the genetic diversity of offspring may function as a “genetic bet-hedging strategy” (Fox and Rauter, 2003; Watson, 1991; but see Yasui, 1998).

Further, the “trading-up” hypothesis (Halliday, 1983) suggests that, by mating with additional males, females may accrue “good genes” benefits for their offspring when their previous mates were of inferior quality (see, e.g., Kempnaers

et al., 1992). The “good sperm” hypothesis (Yasui, 1997) proposes that if there is a correlation between the competitive ability of sperm and offspring viability, polyandry will allow females to obtain indirect fitness benefits for their offspring (see also Hosken et al., 2003). On the other hand, if the competitive ability of sperm is heritable, the “sexy sperm” hypothesis (Keller and Reeve, 1995; Pizzari and Birkhead, 2002) suggests that females that mate multiply will sire sons that themselves are good sperm competitors (see, e.g., Bernasconi and Keller, 2001). Essentially, the trading-up hypothesis is a good genes hypothesis. Correspondingly, the good sperm and sexy sperm hypotheses are the postcopulatory equivalence to the good genes benefit of precopulatory female choice and Fisher’s process of self-reinforcing selection (Andersson, 1994; Fisher, 1930), respectively. All these sexual selection hypotheses are similar in the assumption that genetic effects are additive. Therefore, preferred male genotypes should be the same for all females (Zeh JA and Zeh DW, 2003).

Finally, there is a category of hypotheses known under the term “the genetic incompatibility hypothesis” (Zeh JA and Zeh DW, 1996, 1997). According to this hypothesis, the genetic benefit for females does not depend on males’ intrinsic quality but on the interaction, that is, good fit, between female and male genotype or rather on the good fit between egg and sperm genomes. Genetic incompatibility may be caused by homozygote disadvantage (including inbreeding), selfish genetic elements, and coadapted gene complexes (see Tregenza and Wedell, 2000). The benefit of polyandry rests on the assumption that the females’ eggs are more likely to be fertilized by sperm of more compatible males if females are unable to assess compatibility prior to copulation (Colegrave et al., 2002; Zeh JA and Zeh DW, 1997). Alternatively, females may be restricted in their primary mate choice as being the case in many passerine songbirds. These females may therefore choose to remate with more compatible males (Foerster et al., 2003; Mays and Hill, 2004).

Benefits of polyandry due to genetic incompatibility are difficult to demonstrate (but see Bretman et al., 2004; Tregenza and Wedell, 2002). However, all other hypotheses based on

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genetic benefits of polyandry assume an intrinsic, additive effect of the paternal genome on female fitness. Thus, in a certain environmental context, male genetic quality should be absolute. According to these hypotheses, progeny of different females that have mated with the same male should therefore be of similar viability. Where material benefits and this "intrinsic good genes" prediction can be ruled out, genetic incompatibility has become a recurrent explanation for observations of increased offspring viability due to female polyandry (Newcomer et al., 1999; Tregenza and Wedell, 2000; but see Simmons, 2001).

Females of the scorpionfly *Panorpa cognata* Ramb. mate multiply during their lifetime (Engqvist and Sauer, 2003b). However, in this species, polyandry does not constitute an evolutionary problem as there is good evidence that females receive material benefits from nuptial gift consumption during copulation (Engqvist L, unpublished data). Nevertheless, the verification of direct material benefits of polyandry does not exclude the existence of substantial genetic benefits (see, e.g., Fedorka and Mousseau, 2002). Genetic benefits of polyandry in *P. cognata* were indicated by observations from an abortive heritability study, using a half-sib design with one sire mating with two dams (i.e., all females mated several times with one male). In numerous families, I observed severe failure of egg hatching in several subsequent egg batches. Furthermore, hatch failure or success apparently did not correlate between sire half-sib families, and almost all males were fertile (Engqvist L and Ratering E, unpublished data). Genetic benefits were further investigated in the present study in which female mating partners, but not mating frequency, were experimentally manipulated. I chose to focus on the effects of polyandry on egg-hatching success as this has not only been shown to be strongly affected by female polyandry in similar previous studies (Simmons, 2001; Tregenza and Wedell, 1998) but was also indicated by my initial observation.

Applying an experimental design in which two monandrous females were paired to the same males as a polyandrous female, the additive effect of parental genomes assumed by the intrinsic good genes hypotheses could be estimated. Both intrinsic good genes and genomic incompatibility hypotheses predict polyandrous females to have an increased success in egg hatching. If polyandry benefits are caused by the intrinsic good genes of males, however, one explicitly expects different females mated to one and the same male to have similar egg-hatching rates, whereas this effect is not expected by the genetic incompatibility hypotheses.

MATERIALS AND METHODS

I used F_1 offspring from field-caught adults (near Freiburg in Br., in southwestern Germany) that were bred using standard breeding protocols (Sauer, 1970, 1977; Thornhill and Sauer, 1992). After adult emergence, experimental males and females were held individually in small (8×3.5 cm) plastic tubes and supplied with water ad libitum. All adult scorpionflies used in the experiment were held on a diet consisting of a one-segment piece of a mealworm (*Tenebrio molitor*) every third day.

Essentially, I used the same experimental design outlined in Simmons (2001), which is similar to the one originally used by Tregenza and Wedell (1998). Females were randomly assigned to one of two treatments. Either females mated with two different males (polyandrous) or they mated twice with the same male (monandrous). The experiment was arranged in blocks of three females and two males. One female was mated twice to one of the males, and the second was mated twice to the other male. Finally, the third female was mated twice, once with each of the males. Thus, two females mated monan-

drously and one female polyandrously, but all females mated twice. Individuals in each block were not siblings, but they were of similar age.

Mating trials were staged in plastic boxes ($10 \times 10 \times 7$ cm) containing moist tissue papers and a piece of stem and leaf of a nettle plant (*Urtica dioica*). I ensured that the duration of all copulations was at least 100 min, thereby guaranteeing sperm transfer (Engqvist and Sauer, 2003a). Females were remated the next day, either to the same or the alternate male. These mating trials were repeated each day until the female had mated twice. Male mating order was randomized.

Doubly mated females were provided with a peat-filled petri dish for egg laying and with food ad lib. Boxes were checked daily for eggs laid. If possible, I collected two egg batches of every female. I used a pair of fine and flexible tweezers to carefully transfer egg batches from the egg-laying petri dish to a new petri dish containing moist tissue paper. Egg batches were covered with another layer of moist tissue paper. The number of eggs in each batch was counted, and subsequently batches were incubated at 18°C and an 18:6 h light:dark photoperiod. At these conditions, first instar larvae normally hatch after 8 days. Eggs that had not hatched were incubated for at least twice as long before they were discarded. The number of larvae that hatched was recorded.

Statistical analysis

The data set was grouped into paired observations: the two monandrous females in each block mated with the same males as the polyandrous females. The experiment was performed as two replicates, and I used replicate in the analyses to control for potential random differences. I therefore used repeated measures ANOVAs for the analyses with the mean values of each variable for the two monandrous females and the value for the polyandrous female as paired observations. The test statistics for replicate is reported only if significant. Egg-hatching rates were square root transformed to conform to normality (Lilliefors, $p > .2$), and subsequently data sets did not deviate significantly from the assumptions required by an ANOVA.

In their second copulation, polyandrous females mated with a male to which the female was novel. Monandrous females, on the other hand, were mated to a male that had already mated with the female, hence was familiar with. Differential male allocation of, for instance, sperm and/or seminal fluid to new and previous mates may affect the direct material benefits received by polyandrous and monandrous females. I therefore analyzed whether males show any evidence of distinguishing between novel and familiar females. As only one of the two males in each block mated twice with nonvirgin females, the analysis was based on paired observations for one male within each block. I compared both male mating propensity, measured as the time lag until the second mating, and copulation duration between male matings with nonvirgin novel and nonvirgin familiar females. Not all copulations were observed until their termination, and hence the data set consisted of censored data and was analyzed applying survival analysis. As this analysis included male as a random effect, the parameters of the Cox regression were obtained via penalized estimation using the frailty argument for the coxph function of S-Plus/R (Therneu et al., 2003).

Statistical analyses were performed using SPSS 12.0 except for the survival analysis and the Fligner-Killeen test, which were analyzed using R 2.1.0 (Ihaka and Gentleman, 1996).

RESULTS

Egg-hatching success of females was repeatable across egg batches (coefficient of intraclass variation: $r_i = .502$;

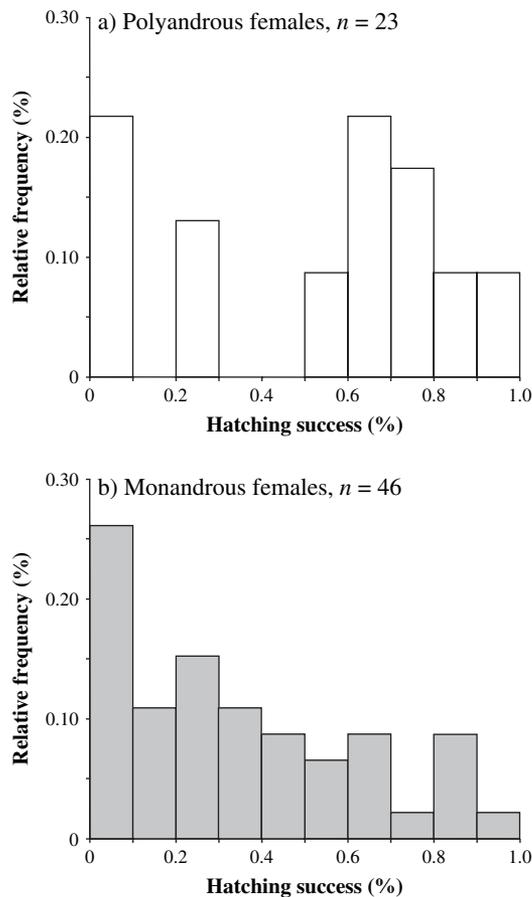


Figure 1
Histogram of egg-hatching success of (a) females that mated with two different males and (b) females that mated twice with the same male.

ANOVA: $F_{77,78} = 3.01$, $p < .001$). The hatching rate of the second batch was not lower compared to the first egg batch of each female (paired t test: $t_{77} = 0.077$, $p = .94$). As a measure of female egg-hatching success, I therefore used the mean egg-hatching rate of both batches for those females, which laid two batches, and the egg-hatching rate of the first egg batch for those females that laid only one egg batch.

In totally 23 blocks, all three females mated twice and laid at least one batch of eggs and were thus statistically evaluable. There was no difference in the mean number of eggs in the first batch laid by the different females (mean \pm SE for polyandrous females: 49.1 ± 5.78 eggs versus 45.9 ± 3.70 eggs for monandrous females; repeated measures ANOVA: $F_{1,21} = 0.212$, $p = .65$). There was also no difference in the time lag between the second mating and egg laying between treatments (repeated measures ANOVA: $F_{1,21} = 0.163$, $p = .69$; statistics performed on log-transformed data). However, eggs from females that mated with two different males had a significantly higher hatching success than those from females that mated twice with the same male (polyandrous versus monandrous: 0.498 ± 0.069 versus 0.335 ± 0.034 ; repeated measures ANOVA: $F_{1,21} = 6.52$, $p = .019$; statistics performed on square root-transformed data; Figure 1). Hatching success of the polyandrous females was even higher, although not significantly, than the hatching success of the monandrous females that mated with the most reproductively successful male within each block (polyandrous versus most successful monandrous: 0.498 ± 0.069 versus 0.369 ± 0.061 ; repeated measures

ANOVA: $F_{1,21} = 3.36$, $p = .081$; statistics performed on square root-transformed data). The frequency of total female reproductive failure was relatively high (16 out of 69 females), but there was no significant difference between treatments (monandrous versus polyandrous: 11/46 versus 5/23; G test: $\chi^2 = 0.041$, $p = .84$).

No correlation between the average hatching success of two monandrous females and the hatching success of the polyandrous females mated to the same males was found ($r = .099$, $p = .65$). I did not find any correlation between the hatching success of the polyandrous female and the hatching success of the female that mated with the most reproductively successful male ($r = .172$, $p = .43$) or the least reproductively successful male ($r = .196$, $p = .37$) within each pair.

Finally, when comparing variances between treatments, I found no indication for a difference between the variance in egg-hatching success of polyandrous females ($n = 23$) and that of monandrous females ($n = 46$) (Levene F test: $F_{1,67}$, $p = .22$, analysis on square root-transformed data; Fligner-Killeen test: $\chi^2_1 = 1.24$, $p = .27$, analysis on untransformed nonnormally distributed data).

Males in their second mating showed no evidence of distinguishing between previous and new mates. First, there was no difference in the propensity to remate, measured as the time lag until the second mating, between matings with novel and previous mates (repeated measures ANOVA: $F_{1,21} = 0.381$, $p = .54$). Second, there was no significant difference in copulation duration of second matings between males mating with novel and familiar females (Cox proportional hazards: -0.385 ± 0.651 , $\chi^2 = 0.35$, $p = .55$). Thus, with respect to female treatment, the observed male mating behavior was similar in all copulations.

DISCUSSION

In this study, I found polyandry to significantly influence female egg-hatching success. Females that mated with two different males had higher egg-hatching success than females that mated twice with the same male. The experimental design rules out any possibility of material benefits from nuptial gift consumption. Moreover, I found that female egg-hatching success was repeatable over successive egg batches. This means that the laboratory conditions were constant enough to rule out the genetic bet-hedging hypothesis (see, e.g., Watson, 1991; Yasui, 1998) as an explanation for the observed difference. Nevertheless, this result alone is in accordance with the genetic incompatibility hypothesis, the good sperm hypothesis, as well as with direct benefits of polyandry due to, for example, infertility assurance (Simmons, 2001; Tregenza and Wedell, 1998).

Let us assume that the increased hatching success of polyandrous females that was found in this study was caused by the heritable superior viability of one of the males and skewed paternity toward this male. In this case, one would expect that the monandrous female that mated with the most viable male within a block should have equal or better hatching success than the polyandrous female within the same experimental block. However, there was no correlation in hatching success between females mated to the same male. Moreover, females mated twice to the most successful male within a block did, on average, worse than the polyandrous females. However, this difference slightly missed statistical significance. Nevertheless, these last results do not coincide at all with the predictions from the hypotheses assuming an intrinsic good genes benefit of polyandry.

Male *P. cognata* differ in their sperm transfer ability (Engqvist and Sauer, 2003a). If the sperm amount transferred by some males is not sufficient to fertilize all of the females' eggs, one

might expect an average difference in hatching success between polyandrous and monandrous females similar to the one found in this study. However, even in this case, one would expect the monandrous female that mated with the most fertile male within a block to have equal or better hatching success than the polyandrous female within the same experimental block. This was not the case, and therefore, fertility differences between males seem unlikely to explain the observed difference in hatching success between polyandrous and monandrous females in this study. Similarly, physical incompatibility between males and females may have caused occasional failure of sperm transfer during copulation. This would affect monandrous females more strongly because only these females would risk to have mated exclusively with a male that failed to transfer any sperm at all due to a bad match between male and female genitalia for instance. However, in *P. cognata*, sperm is always transferred in copulations longer than 1 h (Engqvist and Sauer, 2003a). As copulations in this study always exceeded 100 min, this explanation for differences in egg-hatching rates can be excluded.

The results of this study are in accordance with the predictions from the genetic incompatibility hypothesis. This hypothesis assumes that the viability of offspring will depend on the genetic compatibility of males and females (Jennions and Petrie, 2000; Tregenza and Wedell, 2000; Zeh JA and Zeh DW, 2003). Therefore, one does not expect a correlation in hatching success between females that mated with one and the same male as optimal compatibility will differ between females. Further support comes from the result that polyandrous females within blocks had higher hatching success than the most successful monandrous female.

To date, there are a number of studies that have reported results consistent with the genetic incompatibility hypothesis that polyandry may enhance the viability of embryos due to the avoidance of genetic incompatibility (for review see Tregenza and Wedell, 2000; Zeh JA and Zeh DW, 2003). However, most of these studies are correlative, do not exclude other hypotheses, or do only support some of the assumptions of this hypothesis. So far, the best experimental evidence providing support to the idea that polyandry reduces reproductive failure due to the avoidance of genetic incompatibility comes from studies on crickets (Tregenza and Wedell, 1998) and pseudoscorpions (Newcomer et al., 1999), while other experiments with equal design found no evidence for a genetic incompatibility avoidance benefit of polyandry in bean weevils (Eady et al., 2000) and field crickets (Simmons, 2001, but see below).

A crucial prerequisite for the genetic incompatibility hypothesis, however, is that females are able to bias paternity toward males, or rather sperm, which will be more compatible with the genomes of the females' egg cells (Colegrave et al., 2002; Tregenza and Wedell, 2000; Zeh JA and Zeh DW, 1997). Tregenza and Wedell (1998) found benefits of polyandry in the cricket *Gryllus bimaculatus* equivalent to those in the present study. Following studies have demonstrated that females of this species are able to favor paternity of unrelated males against their siblings (Bretman et al., 2004). Polyandrous females may thus avoid the cost of inbreeding (Tregenza and Wedell, 2002). However, as in the present study, the original study by Tregenza and Wedell (1998) did not entail matings between close relatives. Therefore, the positive effect of polyandry must have had other causes than inbreeding avoidance due to casual matings with close relatives. But after all these studies demonstrate that females have the potential to bias paternity toward more compatible males (see also Bishop et al., 1996; Garner and Schmidt, 2003; Kraaijeveld-Smit et al., 2002; Olsson et al., 1996; Stockley, 1999). Newcomer et al. (1999) found similar effects of polyandry in their study of embryo viability in pseudoscorpions but did not attempt to test the assumption of

paternity bias. Simmons (2001) performed a very thorough analysis of the benefits of polyandry in the field cricket *Teleogryllus oceanicus*. This study demonstrated similar effects of polyandry on female egg-hatching success, but it was argued that the results of further paternal analyses were inconsistent with both the genetic incompatibility hypothesis and the good sperm hypothesis (Simmons, 2001). In this study, there was no a priori expectation of which male of the two should be the more compatible. Therefore, a straightforward paternity assignment of the more compatible male as in the inbreeding experiment in *G. bimaculatus* (see Bretman et al., 2004) was not possible. Nevertheless, one might expect that if females are able to increase hatching success by avoiding fertilizations with incompatible males, there should be a positive correlation between hatching success and paternity skew (see Simmons, 2001). In the study of *T. oceanicus*, no such correlation was found, and the genetic incompatibility hypothesis was therefore excluded as an explanation of the observed benefit of multiple mating (Simmons, 2001).

However, as Tregenza and Wedell (2002) noted, this test has difficulties as there will be variation in the relative compatibility of mates: Females mated to two males of similar high compatibility will have offspring of high viability associated with low paternity skew. Similarly, females randomly mated to two incompatible males may bias paternity toward the slightly more compatible one but still have low offspring viability. In Figure 2, I have simulated the expected correlation between paternity skew and embryo viability if females are able to bias paternity in relation to the relative difference in male compatibility. This illustration shows that it is, as Tregenza and Wedell (2002) pointed out, difficult to test the genetic incompatibility hypothesis in this manner. This association is simply not predicted by the genetic incompatibility hypothesis. Even when there is a strong predicted difference in compatibility between female mating partners, this association may be only weak (Figure 2b). Thus, unless male-female compatibility can be manipulated or estimated beforehand (see Tregenza and Wedell, 2002), paternity assignment does not provide a test of the genetic incompatibility hypothesis. For this reason, I did not attempt to assign paternity in this study.

Nevertheless, Simmons (2001) suggested two alternative explanations for the observed difference in hatching success. First, male ejaculates or equivalents may vary in the quality and composition of gonadotropic substances. To explain the observed effects in the present study requires multiple chemical cues from different males to act synergistically on egg viability. It has been argued that accessory gland products derived from different males may show such an effect on egg production (see Dunn et al., 2005; Eady et al., 2000). Although I am not aware of any study demonstrating such a synergistic effect on egg viability, this possibility cannot be excluded as an explanation of my results. Simmons (2001) further suggested that females may differentially invest in egg cells in relation to male quality (see, e.g., Cunningham and Russell, 2000; Wedell, 1996). Enforced monandry may therefore cause choosy females to withhold resources until they have had the opportunity to mate with multiple males, resulting in lower zygote viability. In this case, one would also expect monandrous females to show a higher reluctance to oviposit or to lay smaller egg batches. I neither observed a difference in egg batch size between treatments nor in the time lag between copulations and egg laying. I therefore conclude that females' differential investment in eggs seems unlikely to account for the observed differences in egg-hatching success.

Female *P. cognata* scorpionflies gain material benefits from multiple mating (Engqvist L, unpublished data). It is therefore easy to understand the selection pressures responsible for the evolution and prevalence of polyandry in this species

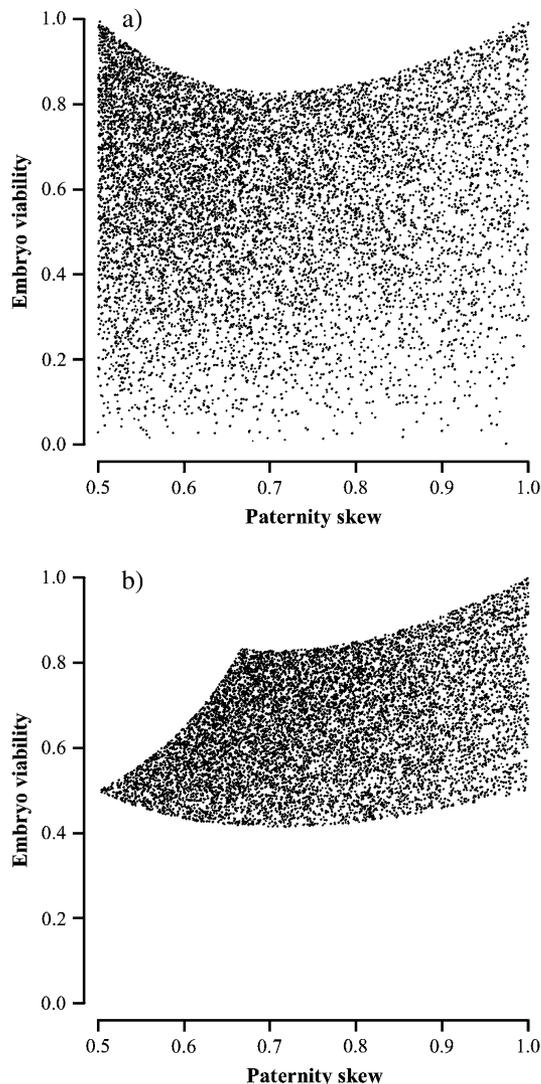


Figure 2

Expected association between paternity skew and offspring viability under the assumptions of the genetic incompatibility hypothesis that females are able to bias paternity toward genetically more compatible males. In (a), 10,000 virtual females were mated to two males (i.e., 20,000 different virtual males). Female-male genetic compatibility (C) equals offspring viability and was assumed to vary uniformly between 0 and 1. It is further assumed that females are able to bias the relative paternity of males (P ; $P_{\text{male}2} + P_{\text{male}1} = 1$) in relation to their relative compatibility with females, hence, $C_{\text{male}2}/C_{\text{male}1} = P_{\text{male}2}/P_{\text{male}1}$. Accordingly, mean female offspring viability will equal $P_{\text{male}1} \times C_{\text{male}1} + P_{\text{male}2} \times C_{\text{male}2}$. In (b), 10,000 virtual females were assumed to mate once with a sibling and once with an unrelated male. Sibling compatibility varied uniformly between 0 and 0.5, and unrelated male compatibility varied uniformly between 0.5 and 1. Note that no residual variance in offspring viability is assumed.

without other benefits. Nevertheless, material and genetic benefits of polyandry are nonexclusive (see Fedorka and Mousseau, 2002). As I have demonstrated, *P. cognata* females are likely to gain nonmaterial benefits of polyandry. This study therefore adds to the currently growing body of evidence emphasizing the importance of genetic benefits of polyandry (e.g., Dunn et al., 2005; Evans and Marshall, 2005; Fedorka and Mousseau, 2002; Garcia-Gonzalez and Simmons, 2005; Ivy and Sakaluk, 2005; Marshall and Evans, 2005; Newcomer et al., 1999; Tregenza and Wedell, 1998, 2002). The genetic

incompatibility hypothesis provides a consistent explanation for the observed difference in egg-hatching success between polyandrous and monandrous females. The results are thus similar to those obtained in equivalent studies (Newcomer et al., 1999; Simmons, 2001; Tregenza and Wedell, 1998). A crucial test of this hypothesis, however, may prove difficult unless male-female compatibilities can be estimated beforehand or independently.

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