



German cockroach males maximize their inclusive fitness by avoiding mating with kin

Mathieu Lihoreau^{a,b,*}, Colette Rivault^{a,1}

^aUMR 6552, Centre National de la Recherche, Université de Rennes 1

^bResearch Centre for Psychology, School of Biological and Chemical Sciences, Queen Mary University of London

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Conventional sex roles imply choosy females and nondiscriminating males. However, growing evidence suggests that male choosiness is more common than expected. While male mate choice is clearly linked to high mating investment in sex role-reversed species, factors promoting male mate choice in conventional sex role species are still debated. We addressed this fundamental issue in the German cockroach, *Blattella germanica* (L.), a group-living species where members of both sexes exercise mutual mate choice to avoid inbreeding. We focused on (1) male potential reproductive rate, (2) male effective reproductive rate, and (3) male reproductive success in relation to female quality. Males tested in situations with unrestrained female availability were able to mate throughout their life, thus revealing a high potential reproductive rate. However, their effective reproductive rate was much more limited under ecologically realistic conditions with restrained female availability. In contradiction with general predictions of sex role theory, mate choice by *B. germanica* males occurs despite an apparent low mating investment and a male-biased operational sex ratio. The finding that inbred matings were less fertile than outbred matings reveals that kinship is the key factor promoting male choice in this particular case. In the light of inbreeding avoidance theory, we propose that by being choosy cockroach males avoid imposing inbreeding costs on their sisters, thus maximizing their own inclusive fitness via kin selection. © 2010 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

A fundamental question raised by the study of sexual selection and mating systems addresses the respective roles of members of each sex in making mating decisions. The ‘classical scenario’ assumes that individuals of one sex are choosy while individuals of the opposite sex compete to gain access to mates. Conventional sex roles traditionally imply female mate choice and male competition. This dichotomy has been the paradigm for a long time because female investment in gamete production and parental care is higher than that of males in the great majority of species (Darwin 1874; Bateman 1948; Williams 1966; Trivers 1972; Andersson 1994). Such asymmetry in resource allocation between males and females leads to lower female potential reproductive rates (i.e. maximum rate of reproduction when not constrained by mate availability) and a male-biased operational sex ratio (i.e. the ratio of males to females available for mating at a given time), which in turn lead males to compete for access to females, and allow females to

choose among males (Emlen & Oring 1977; Clutton-Brock & Vincent 1991; Clutton-Brock & Parker 1992; Vincent et al. 1992; Parker & Simmons 1996). Some reports, although less frequent, also provide evidence for the occurrence of male mate choice and female competition. These cases of reversed sex roles are generally associated with high mating investments by males (e.g. costly ejaculates, paternal care) and female-biased operational sex ratios (e.g. Gwynne 1981; Gwynne & Simmons 1990; Berglund & Rosenqvist 2003).

However, despite its elegant simplicity, this classical scenario does not encompass the breadth of complexity observed. A wealth of information shows that the true spectrum of sexual selection is much broader and that in many species both sexes can compete and be choosy at the same time (e.g. Jones & Hunter 1993; Amundsen & Forsgren 2001; Ryan & Altmann 2001; Saether et al. 2001; Gowaty et al. 2002; Chenoweth & Blows 2003; Drickamer et al. 2003; Preston et al. 2005; Anderson et al. 2007). In recent years, these ideas have been elaborated within an enlarged theoretical framework of mutual mate choice (Parker 1983; Owens & Thompson 1994; Deutsch & Reynolds 1995; Johnstone et al. 1996; Johnstone 1997; Kokko & Monaghan 2001; Kokko & Johnstone 2002; Servedio & Lande 2006) highlighting that at least three key parameters must be taken into account to predict the evolution of

* Correspondence: M. Lihoreau, Research Centre for Psychology, School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Campus, London E1 4NS, U.K.

E-mail address: m.lihoreau@qmul.ac.uk (M. Lihoreau).

¹ C. Rivault is at CNRS UMR 6552, Université de Rennes 1, Campus de Beaulieu Bât 25, Avenue du Général Leclerc, Rennes, 35042 Cedex, France.

mate choice by either sex: (1) mating investment (e.g. parental care); (2) constraints on finding mates and assessing their quality (e.g. operational sex ratio); and (3) quality variance of potential mates (e.g. mate fertility). These evolutionary models converge towards the general prediction that high mating investments, unconstrained mate availabilities and large mate quality variances should favour some degree of mate choice by individuals of one sex. From our point of view, this well-developed theoretical platform is a basis for revisiting mating systems experimentally and shedding new light on our understanding of mate choice by males and females in species with conventional sex roles.

The aim of our study was to identify factors promoting male mate choice in the German cockroach, *Blattella germanica* (L.), a species with conventional sex roles where males and females exercise mutual mate choice to avoid inbreeding (Lihoreau et al. 2008). These gregarious cockroaches live in mixed-family groups, with generation overlaps and a balanced adult sex ratio (Ross & Mullins 1995). Because of their low dispersion rates (Rivault 1990), group members share high levels of relatedness and individuals of both sexes exercise precopulatory mate choice to avoid inbred matings (Lihoreau et al. 2007). Males avoid mating with their sisters by preferentially courting nonsibling females (Lihoreau & Rivault 2009). In turn, females choose to mate with the most vigorously courting males (Lihoreau et al. 2008). After mating, females invest in producing and bearing successive batches of eggs (oothecae). However, there is no evidence for any male parental investment (Rust et al. 1995). Reasons for male choosiness therefore remain unclear.

In the light of recent advances in sex role theory, we addressed this issue by investigating (1) male mating investment, (2) male constraints on finding potential mates and (3) male reproductive success in relation to female quality variance. We first estimated male mating investment by measuring their potential reproductive rates in groups with unbalanced sex ratios and no constraints on female availability (one male in the presence of many females). We then assessed effective reproductive rates of males by testing them under more ecologically realistic conditions in groups with balanced sex ratios. Finally, we evaluated fitness benefits gained by males in relation to female quality variance by comparing the fertility of females in inbred matings to that of females in outbred matings.

METHODS

Experimental Subjects

All experimental subjects came from our *B. germanica* laboratory stock culture. They were reared and tested at 25 ± 1 °C, under an artificial 12:12 h light:dark cycle. Mature oothecae were collected from freely mated gravid females and placed in individual rearing boxes (80 mm in diameter \times 50 mm high) where they hatched. Sibling nymphs remained grouped in these rearing boxes until they became adult. From their imaginal moult to the beginning of tests, adults from a given ootheca were separated by sex to preserve their virginity, but remained in groups to avoid delayed sexual maturation (Lihoreau & Rivault 2008). A colour code (spot of paint on the pronotum) allowed us to discriminate sibling individuals from the same ootheca ($r = 0.5$) from nonsibling individuals from different oothecae ($0 \leq r < 0.5$). These rearing conditions did not affect kin recognition abilities of test cockroaches because relatedness assessment in this species is based on genetically inherited cues (cuticular hydrocarbon profiles), independently of familiarity (Lihoreau & Rivault 2009). Experimental subjects were manipulated under CO₂ anaesthesia prior to experiments. Each individual was tested only once.

Mating Experiments

Potential reproductive rate of males

Experiment 1 was designed to investigate the life-long ability of males to mate and to fertilize females in groups with unbalanced sex ratios. In this nonecologically realistic context, where availability of receptive females was unrestrained and intermale competition was absent, we predicted that high potential reproductive rates (i.e. numerous matings) would suggest a low mating investment by males. In contrast, low potential reproductive rates (i.e. few matings) would indicate a high mating investment.

Immediately after their imaginal moult, test males were placed in individual boxes (140 mm in diameter \times 30 mm high) with a shelter, water and food ad libitum. At the same time, 10 virgin females (7 days after their imaginal moult) that were unrelated to the male (male's nonbrothers) were introduced into each male's box and left there for 25 days (sex ratio = 1:10). This period maximized the females' probability of becoming sexually receptive during the experiment (Lihoreau et al. 2007). To control genetic variability, the 10 females were sisters. After 25 days, females were removed from the boxes of males and kept isolated until the hatching of their oothecae to record their fertility (see *Reproductive success of males*). Old females were then replaced by 10 new virgin sisters. Batches of virgin females were renewed regularly from the time males became adult until their death (male life span range 57–146 days, $N = 30$). As the latency between mating and hatching of the first ootheca is stable in our *B. germanica* strain (mean \pm SE = 24.11 ± 0.31 days, $N = 660$ females), we were able to infer the mating rank of each female (i.e. the order in which they mated with the male) by referring to the hatching date of their first ootheca. This experiment was replicated 30 times.

Effective reproductive rate of males

Experiments 2a–d were designed to investigate the ability of males to mate and to fertilize females in a group with a balanced sex ratio. In this ecologically realistic context, where access to receptive females was restrained and intermale competition was present, we predicted that high effective male reproductive rates (i.e. numerous matings) would indicate low constraints on female availability. In contrast, low effective reproductive rates (i.e. few matings) would indicate high constraints on female availability. Because males do not establish dominance hierarchies or interact agonistically when competing for a female (Breed et al. 1975; personal observations), and because matings occur more often between unrelated individuals than between related individuals (Lihoreau et al. 2008), the quality of males as competitors primarily depends on their relatedness to the females and to the other males in the group.

Males were tested at the beginning of their sexual maturity, estimated at 8 days after their imaginal moult (see experiment 1). On day 7 after their imaginal moult, they were placed individually in plastic Eppendorf tubes. At the same time, groups of four virgin sisters were placed in experimental boxes (140 mm in diameter \times 30 mm high) containing water, food and a piece of cardboard acting as a vertical shelter so that the experimenter could always see the cockroaches. The next day, just before nightfall (i.e. before the beginning of the cockroach activity phase), four males were introduced into each box of females (sex ratio = 1:1). Males were allowed to walk freely from their Eppendorf tubes into the boxes of females, thus avoiding stress caused by CO₂ anaesthesia and manipulation by the experimenter just before the experiment. From then on, we recorded the activity of each group member by direct observations at 30 min intervals, night and day for 25 days. This scan interval allowed us to identify individuals involved in all matings as copulation generally lasts more than 1 h in this species (Ross & Mullins 1995). During the night phase, observations were recorded under red light as it is not perceived by cockroaches

Table 1
Composition of groups tested in experiments 2a–d

Experiment	N	Number of females' brothers	Number of females' nonbrothers
2a	23	4	0
2b	28	3	1
2c	23	2	2
2d	33	0	4

Groups were composed of four males and four sisters. Males were either brothers or nonbrothers of the four females. To control for genetic variability, when several females' nonbrothers were introduced into the groups, they were all siblings. N: number of replicates.

(Koehler et al. 1987). After the 25 test days, mated females were isolated until the hatching of their oothecae to record their fertility (see [Reproductive success of males](#)).

Four experiments (experiments 2a–d) evaluated the reproductive rate of each male in relation to its relatedness with its group members (Table 1). Males were either brothers or nonbrothers of the four females in their group. The percentage of females' nonbrothers within a group varied from 0% (experiment 2a) to 100% (experiment 2d). To control genetic variability, when several females' nonbrothers were introduced into a group, these males were all siblings. To compare reproductive rates of males in relation to their relatedness with their group members, we randomly chose a focal male of each type (either a female's brother or a female's nonbrother) in each group (using function `runif` in R.2.2.1. software, [R Development Core Team 2007](#)). Mean mating rates of focal males were then compared among experiments. Under these conditions, we predicted that (1) the number of matings per male in a group would increase with their opportunities to mate with unrelated females, (2) the reproductive rate of females' nonbrothers would be higher than that of females' brothers in the same group, and (3) the variance in male reproductive rates would be higher in mixed groups containing females' brothers and females' nonbrothers than in groups containing only one type of male.

Reproductive Success of Males

We estimated the reproductive success of males as the total number of offspring they sired in experiments 1 and 2a–d. This involved recording the fertility of each female from her single

mating until her death (range 27–245 days, $N = 929$). All mated females (experiment 1: $N = 611$; experiment 2a: $N = 63$; experiment 2b: $N = 80$; experiment 2c: $N = 66$; experiment 2d: $N = 109$) were maintained isolated in a plastic box (80 mm in diameter \times 50 mm high) and fed ad libitum. The number of fertile oothecae each female produced and the number of viable nymphs hatching from each of these oothecae were recorded daily. After hatching, empty fertile oothecae were opened under a binocular microscope ($\times 25$) to count the remaining nonviable embryos. The sum of viable nymphs and of aborted embryos in a given ootheca gave us the total number of fertilized eggs.

Fertility data from experiment 1 allowed us to evaluate the total number of matings by males, the fertility of each mating in relation to the number of previous matings, and the interval between successive matings. Fertility data from experiments 2a–d allowed us to evaluate the costs of inbreeding by comparing the fertility of females in inbred and in outbred matings. As we never observed multiple matings by females under our experimental conditions, female fertility was an estimate of the reproductive success of a pair.

Statistical Analyses

Data were analysed using generalized linear models (GLMs) and generalized linear mixed models (GLMMs). For normally distributed data (confirmed by a Shapiro normality test), we used an identity link function and assessed the significance of explanatory terms with the F statistic. For Poisson-distributed data (confirmed by a chi-square goodness-of-fit test), we used a log link function and assessed the significance of the explanatory terms with the chi-square Wald statistic. GLMs allowed us to investigate the effects of relatedness and group composition on the reproductive rate of males. We also used GLMs to investigate the effects of relatedness, latency between successive matings, mating rank of females and life span on fertility parameters. We used GLMMs to analyse repeated measures data, including individual's identity as a random factor. This allowed us to investigate the effect of age on male reproductive rates and the effect of ootheca rank on females' fertility. We performed Wilcoxon tests to compare the reproductive rates of females' brothers and females' nonbrothers in each type of group, as well as the fertility parameters in inbred and outbred matings. Differences in the

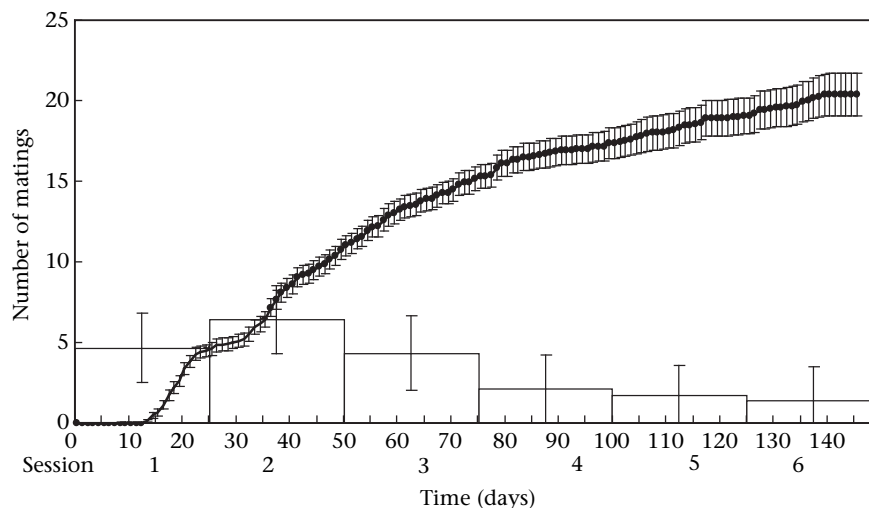


Figure 1. Potential reproductive rates of males without constraints caused by unrestrained availability of receptive females and without intermale competition (experiment 1). Black line: mean cumulative numbers of matings by males \pm SE in relation to time, from their imaginal moult until their death. Bar plots: mean number of matings by males \pm SE in relation to the six successive female batches (session 1–6). Males' mating rates (i.e. number of matings/day) in relation to age: GLMM: $\chi^2_1 = 93.564$, $N = 30$, $P < 0.001$.

variances of male mating rates between group compositions were assessed with a Bartlett test. All statistical analyses were performed using R.2.2.1 (R Development Core Team 2007). Means are given \pm SE.

RESULTS

Potential Reproductive Rate of Males

In experiment 1, in groups with unbalanced sex ratios (1:10), males were presented on average with 47.33 ± 2.44 females during their whole adult life (114.43 ± 5.68 days, $N = 30$). They mated with $43.47 \pm 0.02\%$ of the available females, at a rate of 0.19 ± 0.01 matings per day, meaning that they mated at least once and on average 20.37 ± 1.30 times (Fig. 1). Their mating activity lasted from day 8 after their imaginal moult to shortly before their death (range day 8–day 150, $N = 30$). Mating rates peaked between day 15 and day 55, that is, during the first three female batches, reached a maximum of 4 matings/day, and then decreased slightly with time until the males died (Fig. 1).

Each male sired on average 1789.87 ± 120.10 viable offspring. The total number of fertilized eggs produced by females was significantly influenced by their life span, however; neither their mating rank (i.e. the order in which they mated with males) nor the latency between two successive matings by the males they mated with had any significant effects on the production of fertilized eggs (GLM: female life span: $F_{1,442} = 1020.052$, $P < 0.001$; female mating rank: $F_{33,442} = 1.184$, $P = 0.227$; latency between matings: $F_{1,442} = 3.325$, $P = 0.069$, all interactions $P > 0.05$). Similarly, the total number of viable nymphs (i.e. fertilized eggs that hatched) was influenced by females' life span, but not by their mating rank nor by the latency between two successive matings by the males they mated with (GLM: female life span: $F_{1,442} = 917.663$, $P < 0.001$; female rank: $F_{33,442} = 1.226$, $P = 0.186$; latency between matings: $F_{1,442} = 1.603$, $P = 0.206$, all interactions $P > 0.05$). Therefore, although mating rates of males varied throughout their life (Fig. 1), males seemed to transfer ejaculates of equal quality per mating, whether this occurred several times on the same day when they were young (batches 1–3), or once a week when they were older (batch 6), thus revealing a high potential rate of reproduction. Fertility data also reveal that the total number of fertilized eggs per ootheca

decreased progressively as females aged, between the production of the first and the last ootheca (GLMM: ootheca rank: $F_{6,1701} = 70.381$, $P < 0.001$), while the number of aborted embryos per ootheca increased (GLMM: ootheca rank: $F_{6,1701} = 20.018$, $P < 0.001$). This increase in embryonic lethality highlights an effect of ageing on the process of brood production by females.

Effective Reproductive Rate of Males

In experiments 2a–d, in groups with a balanced sex ratio (1:1), total numbers of matings were similar in all four test situations (2.92 ± 0.10 matings/group; GLM: group composition: $F_{3,103} = 0.562$, $P = 0.643$). None of the females mated more than once (no matings = 110, one mating = 318). Similarly, males rarely mated more than once (no matings = 175, one mating = 197, two matings = 48, three matings = 7, four matings = 1). Males' probability of mating multiply depended both on their relatedness with the females and their relatedness with the other males in the group (Fig. 2). Mating rates of females' brothers did not differ significantly in relation to group composition (GLM: group composition: $\chi^2_2 = 0.090$, $P = 0.956$) and always remained low (0.49 ± 0.07 matings/male). On the other hand, mating rates of females' nonbrothers increased when their proportions in the group decreased (GLM: group composition: $\chi^2_2 = 8.466$, $P = 0.015$) and reached their highest level (1.21 ± 0.23 matings/male) in groups where there was only one nonbrother (experiment 2b). Females' nonbrothers always mated more often than females' brothers when in competition in the same group (Wilcoxon test: experiment 2b: $W = 249$, $N = 28$, $P = 0.012$; experiment 2c: $W = 172$, $N = 23$, $P = 0.029$). As a result, the differences between the mating rates of males in a group were higher in mixed groups containing females' brothers and female's nonbrothers than in groups containing only one type of male, as indicated by the nonhomoscedastic distribution of the variances of mating rates in the four experiments (variance experiment 2a = 0.42, variance experiment 2b = 0.79, variance experiment 2c = 0.52, variance experiment 2d = 0.46; Bartlett test: $\chi^2_3 = 13.08$; $P = 0.004$).

Male Reproductive Success in Relation to Female Quality Variance

All 318 matings observed in experiments 2a–d were successfully fertile. The total number of fertilized eggs produced by females

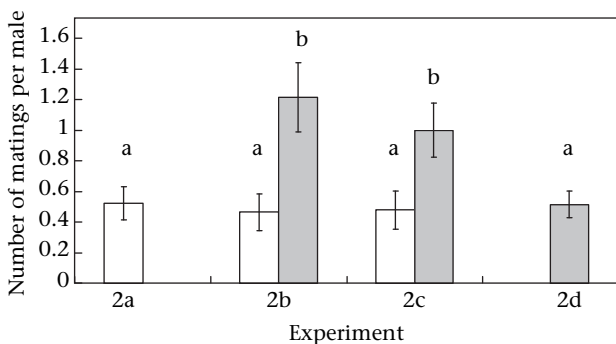


Figure 2. Effective reproductive rates of males under constraints caused by restrained female availability and intermale competition (experiments 2a–d). Males were either brothers or nonbrothers of the four females in their group (see Table 1). Bar plots represent mean numbers of matings \pm SE for females' brothers (white bars) and for females' nonbrothers (grey bars). Males' mating rates (i.e. total number of matings) in relation to their relatedness with other group members: GLM: $\chi^2_2 = 18.98$, $P = 0.002$. Same letters (a, b) above bars indicate no significant differences between numbers of matings (z tests: $P < 0.05$). Number of replicates = 23, 28, 23 and 33 for experiments 2a, b, c, d, respectively.

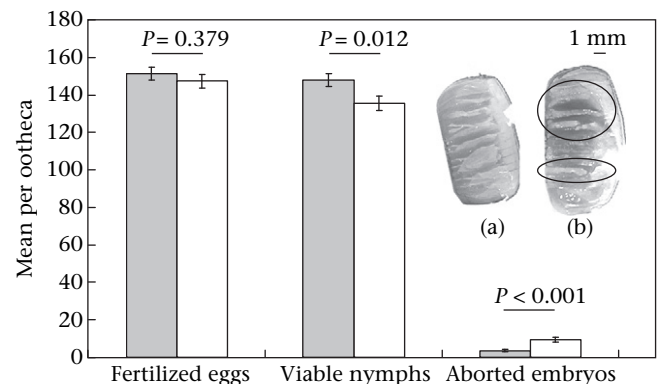


Figure 3. Reproductive success of males in relation to female quality variance (experiments 2a–d). Bar plots show the mean total number \pm SE of fertilized eggs, of viable nymphs and of aborted embryos produced per female in outbred matings (grey bars; $N = 182$) and in inbred matings (white bars; $N = 136$). P : Wilcoxon test. (a) Hatched ootheca that produced only viable eggs (empty compartments). (b) Hatched ootheca that produced both viable eggs (empty compartments) and nonviable eggs (remaining aborted embryos, black circles).

was significantly influenced by their life span, but not by their mating rank nor by the latency between two successive matings by the male, both in outbred matings (GLM: female life span: $F_{1,170} = 3.102$, $P = 0.005$; female rank: $F_{2,170} = 1.365$, $P = 0.184$; latency between matings: $F_{1,170} = 1.054$, $P = 0.302$; all interactions $P > 0.05$) and in inbred matings (GLM: female life span: $F_{1,124} = 14.547$, $P = 0.001$; female rank: $F_{2,124} = 0.572$, $P = 0.567$; latency between matings: $F_{1,124} = 0.033$, $P = 0.857$; all interactions $P > 0.05$). As in experiment 1, these results suggest that males fertilized females with similar quantities of sperm, whatever their mating history. However, although the total numbers of fertilized eggs were similar in outbred and in inbred matings, the total numbers of viable nymphs differed significantly between these two types of matings (Fig. 3). On average, inbred matings produced less viable nymphs than did outbred matings. This difference in fertility is due to a higher proportion of aborted embryos in inbred matings, suggesting the occurrence of inbreeding depression. Again, as in experiment 1, we observed a progressive decrease in the numbers of fertilized eggs per ootheca in relation to female's age (GLMM: ootheca rank in outbred matings: $F_{6,758} = 50.04$, $P < 0.001$; ootheca rank in inbred matings: $F_{6,707} = 18.52$, $P < 0.001$). This was accompanied by a progressive increase in the numbers of aborted embryos per ootheca (GLMM: ootheca rank in outbred matings: $F_{6,758} = 3.53$, $P = 0.002$; ootheca rank in inbred matings: $F_{6,707} = 2.41$, $P = 0.026$). This global reduction in females' fertility caused by ageing was independent of the type of mating.

DISCUSSION

In this study, we demonstrated that mate choice by *B. germanica* males occurs despite their low investment in mating and high constraints on female availability. Unexpectedly, the large female quality variance, related to kinship, seems to play the key role in promoting male choosiness. In the light of inbreeding avoidance theory, we discuss the possibility of a kin-selected mate choice by males that would allow them to maximize their inclusive fitness by avoiding mating with closely related females.

High Potential Reproductive Rate

Our results based on large samples of individuals show that *B. germanica* males are able to mate throughout their adult life when availability of receptive females is not restrained, as suggested by preliminary observations (Ueda et al. 1969; Curtis et al. 2000). Male mating rates peaked during a short period following their sexual maturity and then decreased progressively with age. The novelty of our results is that males' mating success was independent of the number of females they had already mated with and of the latency since their last mating. Throughout their life, males are thus able to court efficiently and to provide ejaculates with sufficient quantities of sperm to allow females to produce fertile oothecae until they die. In contrast to many species in which polygyny induces sperm depletion (e.g. Preston et al. 2001; Jones & Elgar 2004; Harris & Moore 2005), *B. germanica* males' ejaculates were never sperm depleted, as indicated by the absence of abnormally high numbers of unfertilized eggs in females' oothecae. Therefore, in addition to the absence of agonistic competition between courting males (Breed et al. 1975) and the absence of paternal care (Rust et al. 1995), our results suggest that males incur low physiological costs when mating. Courting, production of tergal gland secretions acting as nuptial gifts (Nojima et al. 1996, 1999), production of sperm and production of the nitrogen-rich spermatophore envelope (Mullins & Keil 1980) do not seem to limit their reproduction. This high potential reproductive rate therefore suggests a low mating investment.

Low Effective Reproductive Rate

Our results also confirm that, after a single mating, females store sufficient quantities of sperm to fertilize their entire life-long ootheca production (Lihoreau et al. 2007, 2008). During our experiments, females never remated before the production of their first ootheca. As they never remate between production of successive oothecae (Cochran 1979), there is no doubt that *B. germanica* females mate only once during their lifetime. Consequently, the difference in potential reproductive rates between males and females biases the operational sex ratio towards males and places drastic constraints on their mating opportunities. In our experiments, 41% of the males never mated, 46% mated once, and only 13% remated between one and three times ($N = 428$). Although the variance in mating rates observed in experiments 2a–d may be an underestimate of the real variance expected in natural populations, we believe that the majority of males rarely have the opportunity to mate more than once in an aggregate as they rapidly become less competitive than younger males after day 10–15 of their adult life (Ross 1978; Ross & Mullins 1995).

Mating rates of *B. germanica* males are not the results of intermale precopulatory interactions as confirmed by the absence of agonistic contacts between males that could indicate the establishment of a hierarchy (personal observations), but they are clearly linked to the relatedness of males to the other group members (males and females). As predicted under the hypothesis of male mate choice for inbreeding avoidance, females' nonbrothers had higher mating rates than females' brothers in experiments when they were in direct competition in the same group. Because unrelated males have more opportunities to exercise their mate preference than females' brothers in this situation, they display more vigorous courtships and are chosen more frequently by females. The advantage of female's nonbrothers over females' brothers peaked when they were the minority in the group, as indicated by the high variance of mating rates experienced by males in each type of group. The rarer the unrelated males to females were in a group, the greater their remating opportunities. Given these results, the fact that the total number of mated females remained constant (approximately 75%) whatever the composition of the group may seem counterintuitive. Instead, we would expect it to increase with the number of females' nonbrothers in the group. We suggest that a stable fraction of females remained sexually nonreceptive throughout the experiments, thus limiting the number of mating opportunities for males. Indeed, although all females in a group were sisters of the same age, it is conceivable that manipulations under CO₂ anaesthesia necessary to set up the experiments could have delayed the sexual maturation of some of them. We believe that this small artefact does not bias the main conclusions of the study.

High Costs of Inbreeding

Previous studies demonstrated that *B. germanica* females produced fewer viable nymphs in inbred matings than in outbred matings (Lihoreau et al. 2007, 2008). However, several hypotheses could explain this difference in reproductive success between pairs: (1) a cryptic male choice through differential investment in spermatophore production (Eberhard 1996); (2) a cryptic female choice through differential use of their sperm reserve (Thornhill & Alcock 1983); or (3) inbreeding depression effects in the first generation (Charlesworth & Charlesworth 1987). Our present results allow us to reject the first two hypotheses of cryptic mate choice because females mated only once and because they produced similar quantities of fertilized eggs whatever their relatedness to their mating partner. Instead, the fertility data clearly demonstrate the occurrence of inbreeding depression at the first generation through

a significant increase in embryonic lethality in inbred matings. The quality of females as mating partners is thus closely linked to their genetic relatedness with males. By mating with unrelated females, males avoid inbreeding costs that decrease their direct fitness by 10% at the birth of nymphs.

Conclusions

At first sight, our study highlights a number of results in contradiction with general predictions of sex role theory, and provides evidence for many good reasons for a selection against choosiness in *B. germanica* males. First, the absence of apparent mating costs should always benefit nondiscriminating males over choosy males because they would increase their direct fitness by mating with any encountered female, whether she is related or not. Second, even if a few males manage to mate more than once, the observed high male-biased operational sex ratio introduces important constraints on mate availability. Because receptive females are difficult to find, males would risk never mating at all by rejecting mating with sisters. Then, how can we explain male choosiness in the present case? We propose that our counterintuitive results provide experimental support to an old and somewhat neglected theoretical result showing that choosiness under risk of inbreeding can confer positive effects on the inclusive fitness of parents (Bengtsson 1978; Parker 1979, 2006; Bateson 1983; Waser et al. 1986; Waller 1993; Lehmann & Perrin 2003; Kokko & Ots 2006). By rejecting mating with their sisters, males avoid imposing a fitness cost on closely related females and thus maximize their indirect fitness, owing to the operation of kin selection. Although we observed an embryonic lethality of only 10%, an inbreeding depression much lower than that expected to predict the evolution of such kin-selected mate choice (Parker 1979), Kokko & Ots (2006) recently pointed out that male mate choice becomes much more likely at low inbreeding costs when opportunities to choose are simultaneous rather than sequential. Simultaneous choice introduces an opportunity cost into the system as mating with one female prevents a male from mating simultaneously with another female. We assume that our results could fit this particular case, as males were faced with simultaneous choices in our experiments. Simultaneous choices are also more relevant than sequential choices in *B. germanica*'s natural populations which are generally large enough to provide males with the opportunity to choose to court one of several simultaneously calling females (Lihoreau et al. 2008). For all these reasons, we believe that despite apparent contradictions with the general sex role theory, both costs related to kin selection and mating opportunities may be key factors promoting the evolution of male mate choice in this particular case of inbreeding avoidance. In future manipulations of experimental choice situations (simultaneous versus sequential choices) it would be interesting to assess the relative importance of opportunity costs and inbreeding depression for male choosiness. Because receptive females are more likely to be sequentially encountered in small aggregates (i.e. young populations), we predict that males should be less choosy than in large aggregates (i.e. well-established populations). As illustrated by our results, revisiting mating systems by focusing on both males' and females' mating preferences can provide interesting data to discuss predictions of evolutionary models, a useful approach to develop a robust theoretical framework for the evolution of sex roles.

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