

Simultaneous mating with multiple males reduces fertilization success in the myobatrachid frog *Crinia georgiana*

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Approximately 50% of matings in the frog *Crinia georgiana* involve two or more males. We report reduced fertilization success as a major cost of mating with multiple males. For single-male matings, fertilization success was consistently high averaging 96%. Only 68% of eggs were fertilized when females were amplexed by two males and this dropped to 64% when females were amplexed by three to five males. Multiple regression analysis revealed the reduction in fertilization success was significantly related to the number of amplexant males but not to clutch size or three measures of water quality (depth, temperature and oxygen concentration) at the site of oviposition. The most likely cause of reduced fertilization success is struggles amongst males which interfere with effective sperm transfer.

Keywords: fertilization success; multiple-male mating; *Crinia georgiana*; polyandry

1. INTRODUCTION

Polyandry is broadly defined as a mating system where females mate with multiple males over the duration of a breeding season (Thornhill & Alcock 1983). However, usage of the term varies greatly as a consequence of the diversity of mating patterns among animals. Explanations for polyandry range from forced copulation by promiscuous males (Thornhill 1980) to deliberate choice by females (Zeh 1997). There are a number of arguments about the value of polyandrous mating to females. Mating with two or more males may be an insurance against male infertility (e.g. Sheldon 1994), reduce the risks of sperm depletion (e.g. Bourne 1993), eliminate problems of sperm incompatibility (e.g. Zeh 1997), allow females to select the highest quality sperm (e.g. Olsson *et al.* 1996) or increase genetic variation amongst offspring (e.g. Yasui 1998).

Polyandrous mating, however, may also carry costs, such as increased risk of predation (Arnqvist 1989; Fairbairn 1993), disease transmission (Thrall *et al.* 1997) and reduced foraging efficiency (Sih *et al.* 1990). Recent work on a variety of animal taxa indicates that females may also incur costs resulting from male responses to sperm competition (Stockley 1998). Physical damage may result from mate guarding (Borgia 1981; Davies 1992; Rowe *et al.* 1994; Weigensberg & Fairbairn 1994), prolonged copulations (Thornhill & Sauer 1991; Rowe *et al.* 1994), multiple copulations (Birkhead & Møller 1992; Hunter *et al.* 1993) or transfer of harmful seminal products (Chapman *et al.* 1995). Opportunities for further mating may also be restricted by mate guarding (Davies 1992), the instalment of copulatory plugs (Brock Fenton 1984;

Koprowski 1992), male-induced lack of receptivity (Simmons & Gwynne 1991) or increased mate discrimination by males due to reduced confidence of paternity (Schwagmeyer & Parker 1990; Simmons *et al.* 1994).

Reduced fertility is another potential cost to females. Inter- and intraspecific studies in birds (Koenig 1982; Davies 1985, 1992; Amat 1987) have revealed that the incidence of unhatched and infertile eggs increases with the frequency of conspecific interactions in the mating system. A common explanation for these patterns is that increased competition between males for matings and harassment of females by unmated males both interfere with egg laying and fertilization, but a direct role for interference has not been demonstrated.

For external fertilizers that are not broadcast spawners, close proximity of eggs and sperm as they are released may be critical for achieving high fertilization rates. Among anuran amphibians, several studies have revealed that mismatches in body size, probably leading to poor alignment of the cloacae of males and females, reduce fertilization success (e.g. Licht 1976; Davies & Halliday 1977; Krupa 1988), though sperm depletion may also be involved (see Robertson 1990; Bourne 1993). In the rhacophorid frog *Chiromantis xerampelina* fertilization success is lowered in polyandrous matings if sperm from focal males is experimentally excluded suggesting male position is critical to maximizing fitness (Jennions & Passmore 1993).

In frog species, where several males are simultaneously involved in a mating, interactions between males may prevent males adopting mating positions or sperm release patterns which maximize fertilization success. We tested this prediction by comparing the fertilization success of female *Crinia georgiana* mated naturally with one or two to five males.

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2. METHODS

(a) *Study species*

Crinia georgiana is a small (24–36 mm snout–vent length), myobatrachid frog which is widely distributed in south-western Australia. The species is a nocturnal breeder, with mating activity occurring continuously between mid-autumn and late spring (Main 1965). Males aggregate in areas of shallow water and vocalize to attract females (Ayre *et al.* 1984). Females attracted to the chorus usually select a mate and the pair enter into inguinal amplexus (where the male mounts the female dorsally and embraces her in front of the hind legs). During amplexus females release eggs which are fertilized externally. The eggs are large and generally deposited in discrete clumps (Seymour & Roberts 1995).

Roberts *et al.* (1999) showed that polyandrous matings (i) occur when non-paired males join an amplexed pair, (ii) are common, occurring in approximately half of all matings, (iii) result in multiple paternity of a clutch of eggs, and (iv) second males typically clasp the female ventrally, while additional males clasp the female dorsolaterally.

(b) *Study site and procedure for locating mating assemblages*

Field work was conducted at Kangaroo Gully, 30 km east–south-east of Perth, Western Australia. The 600 m² breeding site was a series of shallow, seasonal pools on a sloping, moss-covered, granite outcrop surrounded by low scrub and forest. Work was conducted between 18.00 and 03.00 from 12 June to 28 October 1997.

Mating assemblages were located by searching the chorus. Mating frogs were observed until egg deposition was complete. The number of males mating and the positions of males relative to the female were recorded. For all procedures below, frogs were weighed to the nearest 0.1 g using a digital balance.

(c) *Clutch collection and determination of fertilization*

When egg deposition was finished the whole clutch was collected and eggs were placed in a ventilated plastic container. On return to the laboratory eggs were transferred into Petri dishes containing 5 ml of distilled water, which partially covered them. Developing eggs were stored overnight in a constant temperature room at 15 °C. Every egg in a clutch was examined within 24 h of collection and only cleaving eggs were counted as fertilized (cf. Bourne 1993). The percentage of eggs fertilized was compared by Kruskal–Wallis ANOVA with the number of males (one, two or more than two) as factors. Differences in variance between the groups were tested on arcsine-transformed data using Levene's analysis.

(d) *Effect of water quality and clutch size on fertilization success*

Factors other than the number of amplexant males may affect fertilization success. For each mating, we measured the depth, temperature and oxygen tension of the water at the oviposition site because we presumed these may affect fertilization success, e.g. by altering sperm motility. Temperature was measured with a Miller and Weber cloacal thermometer, water depth with a plastic ruler (1 mm graduations) and oxygen tension (ml O₂ l⁻¹) with a WTW OXI 320 oxygen meter. We also recorded clutch size (total number of eggs) because fertilization success may be reduced if sperm-depleted males attempt to

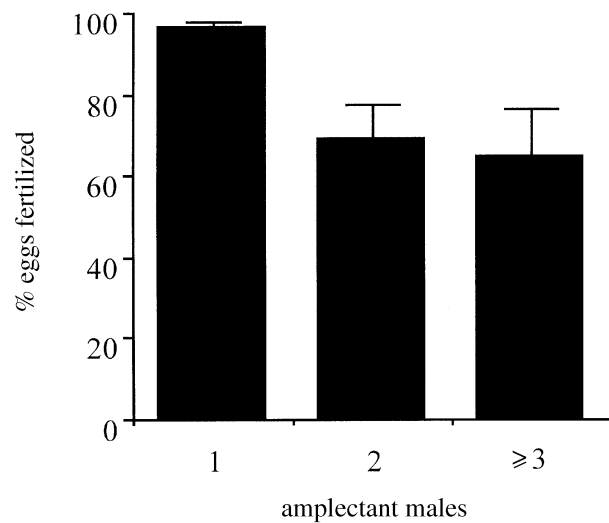


Figure 1. Percentage of eggs fertilized for matings involving one, two and more than two males. The vertical bar is the standard error.

fertilize large clutches (cf. Bourne 1993). Multiple regression analysis was used to examine the importance of these factors relative to the number of amplexant males.

(e) *Effect of body size ratio on fertilization success*

The influence of the body size ratio of mating pairs was also tested because this has been shown to affect fertilization success (e.g. Bourne 1993). Fertilization success was determined for naturally paired frogs in single-male matings. Matings were also staged in the laboratory using males that were smaller, roughly equal to and larger than females. Females were collected from field choruses and paired with calling males from the same chorus. Matings were staged in 5 mm of water, with cotton towel as substrate, in opaque plastic containers measuring 16.5 cm × 15 cm × 9 cm. A male and a female were introduced between 23.00 and 03.30 without illumination and left together undisturbed for *ca.* 10 h. Clutches were then collected and the percentage of eggs fertilized determined.

Relationships between body size ratio (male body weight/female body weight) and fertilization success were analysed using polynomial (quadratic) regressions to detect optimal fertilization success ratios (cf. Bourne 1993).

3. RESULTS

(a) *Field fertilization success*

Egg clutches were collected from a total of 34 matings: 20 involved single males, ten involved two males, three involved three males and one involved five males. Because the sample sizes for matings that involved three and five males were low they were grouped together for statistical comparison.

Fertilization success was significantly affected by the number of males involved in a mating (corrected for ties, Kruskal–Wallis $H=14.96$ and $p<0.001$; figure 1). When females mated with one male the mean (\pm s.e.) percentage of fertilized eggs was consistently high: 96.43% (\pm 1.38). When females mated with two or more males, fertilization success decreased to 68.78% (\pm 8.81 for two males) and 64.59% (\pm 11.81 for more than two males) and there was a large increase in variance among clutches

Table 1. Multiple regression coefficients \pm standard error for factors that might influence fertilization success in eggs of the frog *C. georgiana*

($n=20$: data from nine single-male and 11 multiple-male matings.)

variable	coefficient	<i>t</i>	<i>p</i>
number of males	-12.83 ± 4.49	2.85	0.01
water depth (mm)	-1.00 ± 0.52	1.89	0.08
water temperature ($^{\circ}\text{C}$)	-3.23 ± 2.24	1.43	0.17
oxygen concentration ($\text{ml O}_2\text{l}^{-1}$)	2.41 ± 2.53	0.95	0.35
clutch size	0.08 ± 0.13	0.59	0.56

Table 2. Water quality measures and clutch size for single-male and multiple-male matings

(Values are means \pm standard error (sample size). No means differed significantly (*t*-test, $p=0.05$). Data were from all matings observed where we had partial data ($n=34$), not the limited set with complete data used in the multiple regression analysis ($n=20$).

variable	single	multiple	<i>t</i>	<i>p</i>
number of males	1 (20)	2.4 ± 0.23 (14)	—	—
water depth (mm)	10.8 ± 1.11 (12)	10.0 ± 2.08 (13)	0.23	0.82
water temperature ($^{\circ}\text{C}$)	9.4 ± 0.65 (12)	10.2 ± 0.64 (11)	0.93	0.36
oxygen concentration ($\text{ml O}_2\text{l}^{-1}$)	9.0 ± 0.76 (9)	8.7 ± 0.44 (12)	0.40	0.68
clutch size	59.8 ± 7.43 (20)	74.4 ± 6.77 (14)	1.41	0.17

(Levene's test $F_{2,31}=4.422$ and $p<0.05$) (figure 1). Matings involving one male had significantly higher fertilization success than all other matings. Differences between matings involving two and more than two males were not significantly different ($p>0.05$; *post-hoc* comparisons for Kruskal–Wallis from Zar (1984, p. 200)).

(b) Effect of water quality and clutch size on fertilization success

Multiple regression analysis revealed that clutch size and the depth, temperature and oxygen tension of water at the oviposition site, together with the number of amplexant males, explained a significant amount of the variation in fertilization success in *C. georgiana* ($r^2=0.6065$, $F_{5,19}=4.31$ and $p=0.014$). However, only the number of amplexant males made a significant contribution to the multiple regression model (table 1). There were no significant differences in mean values for oxygen tension, water temperature, water depth and clutch size for monandrous and polyandrous matings (table 2).

(c) Effect of body size on fertilization success

Field data for 19 single-male matings showed no evidence of an optimal body size ratio giving high fertilization success (figure 2a; quadratic regression $F_{2,16}=3.2$, $p=0.07$, $r^2=0.286$ and $n=19$). For 15 matings in the laboratory, fertilization success did not peak significantly (figure 2b; quadratic regression $F_{2,12}=2.67$, $p=0.11$,

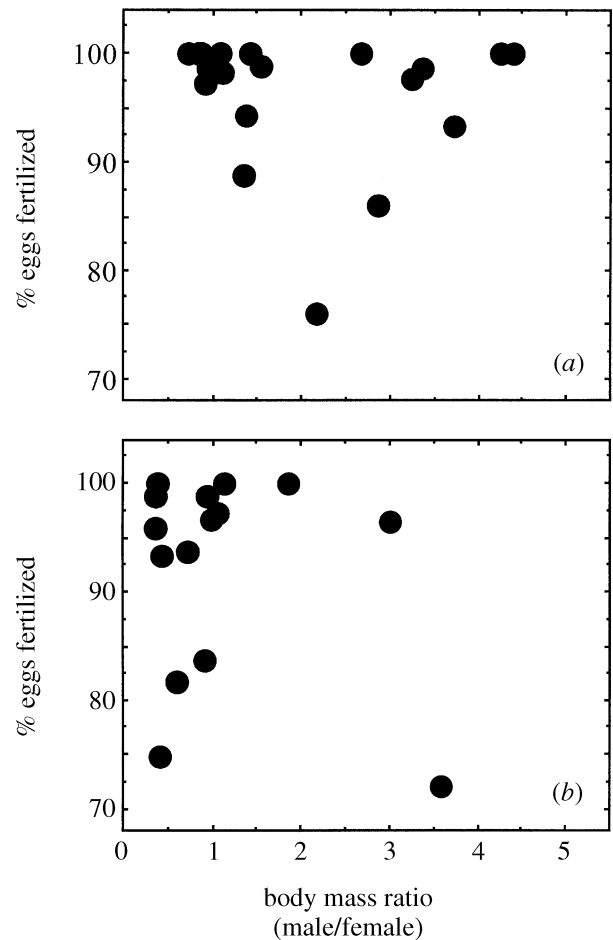


Figure 2. Percentage of eggs fertilized versus ratio of male to female body mass. (a) Field data (lowest ratio 0.71: female 1.4 g and male 1.0 g, and highest ratio 4.4: female 1.0 g and male 4.4 g; lowest fertilization success 76%: female 1.2 g and male 2.6 g). (b) Laboratory data (lowest ratio 0.35: female 3.5 g and male 1.2 g, and highest ratio 3.5: female 1.6 g and male 5.9 g; lowest fertilization success 72.2%: female 1.6 g and male 5.9 g).

$r^2=0.308$ and $n=15$). The contrasting patterns in the field (low at body size ratio of 2) and laboratory data (peak at body size ratio of 2; figure 2) suggest no consistent effect of body size ratio on fertilization success. The average fertility under laboratory conditions was slightly lower than under natural conditions, though not significantly so (laboratory mean = 92.25% (± 2.42) and field mean = 96.43% (± 1.38); $t_{32}=1.58$ and $p=0.12$).

4. DISCUSSION

As predicted, multiple-male mating reduced fertilization success in *C. georgiana*. For females who mated with single males, mean fertilization success was consistently high (figure 1) and comparable with the 90–95% level reported for other anuran species where females mate with single males (e.g. Davies & Halliday 1977; Howard 1978; Kruse 1981; Ryan 1985). The involvement of extra males in a mating, however, led to significant reductions in fertilization success.

In single-male matings, reductions in fertilization success were not related to the body size ratio of mating

pairs, indicating that specific body size relationships are not required to achieve high fertilization success in *C. georgiana*. The results from polyandrous matings are unlikely to be due to any simple problem of mismatch in body size. Decreased fertilization success can most likely be attributed to interference resulting from intense inter-male conflict.

When intruding males join mating pairs violent struggles typically ensue. Most fighting results from attempts of unpaired males to force a takeover and resistance of the paired male to these attacks. Struggles are usually resolved when unpaired males amplex the female ventrally or in other suboptimal positions. However, there is often continued jostling throughout egg deposition as the males attempt to gain cloacal proximity (P. G. Byrne, unpublished data). Such interactions may reduce fertility in several ways. First, intruding males may physically obstruct sperm transfer of the paired male. Second, fighting may prevent any male from adopting an optimal mating posture and obtaining cloacal apposition throughout the entire duration of egg deposition. Third, males may stop releasing sperm during combat. Further work is required to discriminate between these possibilities.

Sperm allocation models have been developed in the context of limits on sperm production and repeat mating interval (Shapiro & Giraldeau 1996) and the risk and intensity of sperm competition (Parker *et al.* 1996, 1997). Incorrect sperm allocation strategies by males may reduce total sperm expenditure per egg. For example, under the Parker *et al.* (1996) models, the amount of sperm released in a single-male mating is low but sufficient to fertilize all eggs. Sperm numbers peak for two males in sperm competition but then decline with increasing numbers of males. If focal and ventral males allocate sperm based on the number of males amplexed (greater than two) but other males do not actually release sperm, it is possible that too few sperm are released to fertilize all eggs.

Sheldon (1994) argued that fertility may be related to male phenotype and success in obtaining extra-pair copulation in birds. If all males involved in multiple-male matings were of comparatively poor phenotypic quality this might affect their ability to fertilize eggs. We have no data on male quality.

Irrespective of the specific cause, reduced fertility is a major fitness cost for females. Such costs suggest females would be expected to actively avoid polyandrous matings or protest against the involvement of secondary males. Because females do not exhibit any such behaviour, the possibility that they are trading reduced fertility against other benefits must be considered. Polyandry may generate compensating benefits by (i) acting as a bet hedge against a partner having low-quality ejaculates (cf. Bourne 1993; Sheldon 1994; Olsson *et al.* 1996; Zeh 1997), (ii) enhancing the genetic diversity of progeny (Yasui 1998), or (iii) reducing or avoiding harassment by persistent males (Stockley 1998).

It is extremely difficult to conceive, however, that any benefits accrued via such means could offset the costs associated with the fertility reduction we have documented. Moreover, females risk death by being involved in multiple-male matings. Over two breeding

seasons there was a *ca.* 2% chance in polyandrous matings that a female would be asphyxiated by competing males (P. G. Byrne, unpublished data).

Simultaneous mating with multiple males may be a case of sexual coercion. If so, lack of protestation by females may be a mechanism to avoid injury or death or occurs because resistance is ineffective. However, until compensatory benefits have been investigated we cannot dismiss the possibility that polyandry is an adaptive female strategy.

Although both sexes have a reproductive interest in the completion of mating, the tactics employed to achieve this may conflict. Within *C. georgiana* choruses, male density is typically very high and the operational sex ratio is heavily male biased (P. G. Byrne, unpublished data). Such extreme conditions are likely to place a strong selective pressure on unattractive males to force copulations at the expense of females. The total testes mass relative to body mass of *C. georgiana* is much larger than 13 other *Crinia* species (mean 1.1% for *C. georgiana* and range of means for 12 other species: 0.03–0.28%; P. G. Byrne, unpublished data) indicating that simultaneous polyandry and sperm competition may have had a long evolutionary history in this species. This mating system is analogous to those of several bird species where harassment of females by unmated males is the likely cause of unhatched and infertile eggs (Koenig 1982; Davies 1985, 1992; Amat 1987). Costs in avian breeding systems are variable (less than 3.3% for duck species (Amat 1987), but up to nearly 20% for some cooperatively breeding birds (Koenig 1982; Davies 1985)), but in most cases the causes of fertility loss are not clear (cf. Birkhead *et al.* 1995). Simultaneous polyandry coupled with external fertilization may be more sensitive to direct interference from competing males than mating systems with internal fertilization. Investigations of the cost of polyandry in other group spawners (e.g. Brockman *et al.* 1994; Taborsky 1998) is required to determine whether reduced fertilization success is unique to *C. georgiana* or whether it is a common and overlooked cost of polyandry in external fertilizers.

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