

Post-mating sexual selection increases lifetime fitness of polyandrous females in the wild

Diana O. Fisher¹, Michael C. Double¹, Simon P. Blomberg², Michael D. Jennions¹ & Andrew Cockburn¹

Females often mate with several males before producing offspring¹. Field studies of vertebrates suggest, and laboratory experiments on invertebrates confirm, that even when males provide no material benefits, polyandry can enhance offspring survival^{2,3}. This enhancement is widely attributed to genetic benefits that arise whenever paternity is biased towards males that sire more viable offspring^{1,4,5}. Field studies suggest that post-mating sexual selection biases fertilization towards genetically more compatible males^{6,7} and one controlled experiment has shown that, when females mate with close kin, polyandry reduces the relative number of inbred offspring⁸. Another potential genetic benefit of polyandry is that it increases offspring survival because males with more competitive ejaculates sire more viable offspring⁹. Surprisingly, however, there is no unequivocal evidence for this process¹⁰. Here, by experimentally assigning mates to females, we show that polyandry greatly increases offspring survival in the Australian marsupial *Antechinus stuartii*. DNA profiling shows that males that gain high paternity under sperm competition sire offspring that are more viable. This beneficial effect occurs in both the laboratory and the wild. Crucially, there are no confounding non-genetic maternal effects that could arise if polyandry increases female investment in a particular reproductive event¹⁰ because *A. stuartii* is effectively semelparous. Our results therefore show that polyandry improves female lifetime fitness in nature. The threefold increase in offspring survival is not negated by a decline in maternal lifespan and is too large to be offset by an equivalent decline in the reproductive performance of surviving offspring.

Field studies show that the offspring of polyandrous females often have lower juvenile mortality². Experiments that manipulate the number of mates while controlling for mating frequency confirm that this is directly due to polyandry^{3,10}. These experiments are largely restricted to laboratory studies of invertebrates¹⁰. It is unclear whether polyandry has an equivalent effect in the field, because juvenile survival depends heavily on the interaction between investment in growth and environmental conditions¹¹. More importantly, these experiments involve species with a breeding biology that makes it difficult to distinguish between competing explanations for how polyandry improves offspring survival or, ultimately, net fitness.

First, females paired with preferred males often invest more in reproduction¹². Similar differential allocation could occur if females increase investment in reproductive bouts when they have more mates¹⁰. These maternal effects could account for the higher offspring survival of polyandrous females. Four studies that directly tested for maternal effects showed that these effects contributed to polyandry improving offspring performance^{11,13–15}. This makes it problematic to conclude that polyandry is beneficial solely due to genetic benefits arising from greater fertilization by sperm carrying genes that increase juvenile survival. Second, paternity tests on older offspring

could create a spurious correlation between estimated fertilization success and offspring viability if early mortality varies among the progeny of competing males¹⁰. One should therefore assess paternity shortly after fertilization, or use mortality rates of offspring of known parentage to correct, retrospectively, initial estimates of paternity. Third, polyandry confers genetic benefits only if it increases net offspring fitness. There is evidence that males with more competitive ejaculates sire offspring with higher values for specific fitness components, but these components do not include survivorship^{16,17}. More importantly, there is no clear evidence linking net offspring fitness to ejaculate competitiveness because there are plausible life history trade-offs with unmeasured fitness components¹.

Here we have investigated how polyandry affects offspring survival in the brown antechinus *Antechinus stuartii*. The unusual life history of this Australian marsupial enables us to resolve these problems. The annual mating season lasts 10–14 d, during which females mate multiply and then give birth synchronously. Paternal care is absent because males die before offspring are born¹⁸. Crucially, ~93% of females breed once because survival between years is extremely low. Females should therefore invest maximally in every litter. Gestation lasts ~27 d, but embryos remain in a suspended state and implant only 4–5 d before birth¹⁹. Unlike placental mammals, young are born as early stage embryos (mass, ~0.016 g) and fuse obligatorily to a teat for 35–40 d before detaching. Young are weaned after ~90 d (ref. 20). The average female produces 8% more young than can attach to her 8–10 teats and the excess die¹⁹. We can assign paternity and monitor survival from birth.

We conducted two separate experiments. In 2003, we trapped antechinuses from a natural population shortly before the mating period. In the laboratory, we assigned 17 females to a polyandry (three males per female) and 19 to a monandry (one male per female, three times) mating treatment. The proportion of females with young attached to every teat (see Supplementary Information) was higher for polyandrous females ($\chi_1^2 = 8.0$, $P = 0.005$). There was no early offspring mortality before releasing females at the original study site after ~35 d; however, the proportion of each litter that then survived to weaning was threefold greater for polyandrous females ($\chi_1^2 = 21.9$, $P < 0.0001$; Fig. 1). There was no subsequent effect of mating treatment on the survival of the remaining offspring to the next breeding season (Jolly–Seber estimates at 4-week intervals: polyandrous, 0.83 ± 0.05 (mean \pm s.e.m.); monandrous, 0.84 ± 0.05).

In 2004, we replicated the experiment but kept lactating females in captivity until just before weaning. We therefore determined whether the previous benefit of polyandry depended on stressful natural conditions, or was replicable in a benign laboratory environment (*ad libitum* food). We also calculated the relationship between the share of paternity of a male and the survival of his offspring. We used independent data (different females) to measure each parameter in a blocked design with 48 wild-caught females and 24 wild-caught

¹School of Botany and Zoology, and ²Centre for Resource and Environmental Studies, Australian National University, Canberra, Australian Capital Territory 0200, Australia.

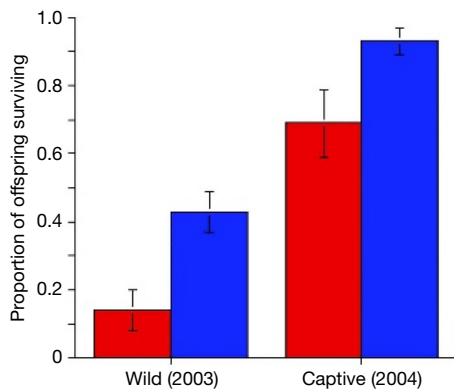


Figure 1 | Effect of mating treatment and rearing environment on the proportion of offspring surviving to weaning. Survival in the wild is estimated from the number of offspring that were captured at weaning. We released 100 offspring from 17 monandrous females and 139 offspring from 19 polyandrous females into the wild. Survival in captivity is calculated from the number of offspring that survived until weaning. There were 121 offspring from 18 monandrous females and 116 offspring from 18 polyandrous females in the captive experiment. Red bars indicate monandry; blue bars indicate polyandry. Data are the mean \pm s.e.m.

males. In a block, each of three males mated once to three different polyandrous females (systematically varying male mating order) and three times with one of three monandrous females. Although the effect of mating treatment did not differ between years (Likelihood ratio test: $\chi_1^2 = 2.5$, $P = 0.11$), polyandry had no effect on the proportion of females that had young attached to every teat in 2004 ($\chi_1^2 = 0.54$, $P = 0.46$). The effect of polyandry on the proportion of females that had young attached to every teat is therefore equivocal (both years: polyandry, 0.59 ± 0.08 ; monandry, 0.32 ± 0.07).

Captive females had significantly lower offspring mortality than those released into the wild ($\chi_1^2 = 146.2$, $P \ll 0.001$); however, offspring survival to weaning was still higher for polyandrous females ($\chi_1^2 = 4.0$, $P = 0.046$; Fig. 1). In fact, the offspring viability benefit of polyandry was the same for captive and wild females ($\chi_1^2 = 0.4$, $P = 0.53$), so there was no detectable effect of the rearing environment. The mating treatment effect was due to a sudden increase in offspring mortality for monogamous females after 65–70 d (Fig. 2).

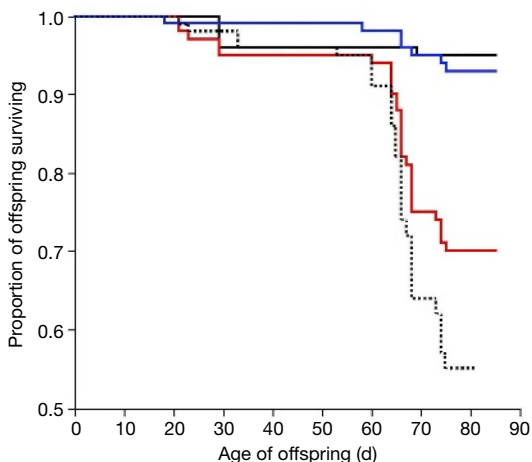


Figure 2 | Effect of mating treatment and male ejaculate competitiveness on survival of offspring in captivity from birth to release (2004). Mean survival curves are presented for monandrous females mated to more competitive males ($n = 41$ offspring from five litters; unbroken black line) or less competitive males ($n = 80$ offspring from 13 litters; dotted black line) and polyandrous females ($n = 116$ offspring from 18 litters; blue line). The pooled curve for all 18 monandrous females that gave birth is also shown (red line).

We counted the total number of offspring that each male sired with three polyandrous females to estimate his ejaculate competitiveness. The survival of offspring produced when females mated monandrously increased strongly with the number of offspring sired by their mate in the polyandrous treatment ($\chi_1^2 = 36.8$, $P \ll 0.001$). We also divided males into two categories: those with less competitive and more competitive ejaculates (0–7 versus 9–15 offspring; Fig. 3). Females that were assigned a more competitive male did not have young on every teat more often than those assigned a less competitive male ($\chi_1^2 = 2.1$, $P = 0.15$). However, offspring survival to weaning was far higher for females that mated with a more competitive than a less competitive male (Wald test: $\chi_1^2 = 4.20$, $P = 0.019$), and no different to that of polyandrous females ($\chi_1^2 = 0.002$, $P = 0.49$; Fig. 2).

This study shows that genetic benefits from polyandry increase female fitness in nature in the absence of male provisioning effects and that this effect can be replicated in captivity. We can eliminate confounding non-genetic maternal effects because semelparity disfavours deferment of reproductive investment, and offspring actually died while mothers were still freely lactating. The threefold increase in the probability of offspring surviving to weaning is unlikely to be negated by unmeasured fitness costs. We can dismiss an effect of polyandry on lifetime litter production as most females breed once²⁰, and mating treatment did not affect maternal survival between years (Fisher's exact test, $P = 0.81$). There was also no detectable trade-off between early and late offspring mortality as polyandrous and monogamous females had almost identical post-weaning offspring survivorship. Although we did not directly determine offspring lifetime fitness, it is highly improbable that unmeasured life history trade-offs are sufficiently strong to eliminate the threefold increase in offspring survival. A negative effect of polyandry on offspring reproductive success is unlikely. Male success is determined largely by body size^{18,21,22} and female fecundity in this study was unrelated to body size ($P = 0.98$, $n = 86$). As seen in a closely related species²³, the offspring of polyandrous females were actually slightly bigger ($\chi_1^2 = 6.2$, $P = 0.01$; see Supplementary Information). A possible caveat is that sexually antagonistic genes might increase the fitness of sons and reduce that of daughters. However, mating treatment did not differentially affect the survival of sons and daughters ($\chi_1^2 = 0.26$, $P = 0.61$; see Supplementary Information).

It has been argued, but not yet shown¹⁰, that polyandry confers genetic benefits because paternity is biased towards sires that generally produce more viable offspring ('good sperm' hypothesis)⁹. In *A. stuartii*, the ability of a male to gain a high share of paternity with three different polyandrous females was positively related to the survival of his offspring when he mated to a fourth female. Although we did not assign paternity at conception, we determined it ~ 6 d after embryo implantation. This reduces the likelihood that differential

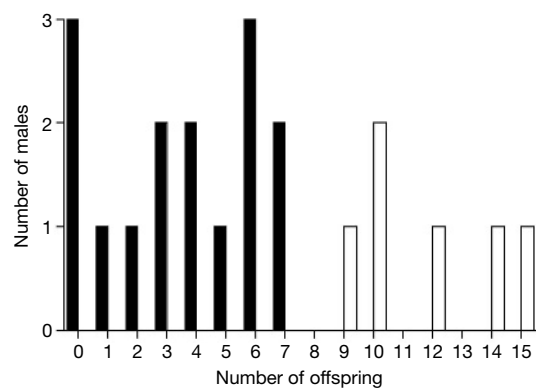


Figure 3 | Total number of offspring sired by males when mated to three polyandrous females. In the 2004 captive female experiment, males with 0–7 offspring were assigned to the 'low ejaculate competitiveness' category (filled bars) and those with 9–15 offspring to the 'high competitiveness' category (open bars).

mortality created a spurious relationship between offspring survival and estimated ejaculate competitiveness¹⁰. Moreover, there was negligible mortality in the first 35 d after birth, differential offspring mortality only occurred shortly before weaning, and sire ejaculate competitiveness was unrelated to litter size at birth. Because our estimates of the ability to gain paternity and the sire effect on offspring survival were independent, our results strongly support the 'good sperm' hypothesis.

In some species, polyandry reduces the production of inbred young^{2,8}, but this is unlikely in *A. stuartii*. Our experimental design precluded females from mating with close relatives (see Supplementary Information). However, our data do not shed light on whether polyandrous *A. stuartii* females can also bias paternity to reduce incompatibility between parental genotypes¹⁰. 'Genetic compatibility' and 'good sperm' explanations for polyandry are not mutually exclusive. The former requires non-additive genetic variation and the latter additive genetic variation for fitness²⁴. Both sources of variation can be significant simultaneously²⁵. Of course, both hypotheses also require that post-copulatory mechanisms can bias paternity in the appropriate direction^{1,4,5,10,24}. Our study shows that polyandry benefits female *A. stuartii* because males that sire fitter offspring gain more paternity under sperm competition. The strength of our results may be due in part to the unusual reproductive biology of this species. In *A. stuartii*, total spermatogenic failure occurs about 1 month before mating, and sperm are continually lost in the urine through spermatorrhoea. Male antechinus copulate for 5–14 consecutive hours with each female, and ejaculate around 3 h after mating starts^{18,21}. This extraordinary male reproductive biology could subject sperm to extreme physiological and epigenetic stress, resulting in the marked relationship between male sperm competitive ability and offspring viability (see Supplementary Information).

METHODS

Mating. We caught antechinus at Kioloa, Australia (35° 32' S, 150° 22' E) in August 2003 and 2004, shortly before the predictable onset of mating and transferred them to the laboratory²¹. We detected oestrus by daily monitoring of urine for cornified epithelial cells²³. Receptive females were alternately assigned to the two mating treatments to standardize the date of mating. In 2003, the 19 females in the monandrous treatment each mated three times to a uniquely assigned male and the 17 females in the polyandrous treatment each mated to three different males. We assigned mates from a pool of 41 males so that the cumulative number of prior matings did not differ between treatments. Females mated every second day. In 2004, we used a blocked experimental design. Each block had six females and three males. The three polyandrous females each mated once to each male such that each male mated once as the first, second and third mate (that is, ABC, BCA and CAB). The three monandrous females each mated three times to a single male (that is, AAA, BBB and CCC). Males therefore mated six times. Again, the cumulative number of prior matings per male did not differ between treatments. No antechinus mated more than once a day. After each mating, we confirmed that insemination was successful by examining female urine or a copulatory plug for the presence of sperm. This design provided independent estimates of the ability of a male to gain paternity and his effect on offspring survival.

Offspring paternity and survival. Females were housed individually and fed *ad libitum*²³. We checked pouches daily for young from 27 d after the first mating. In 2003, we measured the crown–rump length of each 32-d-old offspring, gave it a toe-bud clip and then, at 34 d old, released the family into a nestbox at the point of original capture. To calculate survival to weaning, we directly counted young in the nestbox when they were ~80–85 d old ($n = 12$ families) or, if the family had moved to a natural cavity nearby ($n = 36$), we intensively trapped outside the nest to catch young making initial exploratory forays²⁰. After weaning at ~85–111 d old, we also comprehensively trapped the site every fourth week until the following breeding season²⁰. Recaptured offspring were individually micro-chipped. In 2004, females were checked every 3 h (7 h overnight) from 27 d after their first mating to collect any offspring that failed to attach for genotyping. All young were genotyped (see Methods in refs 23, 26, and Supplementary Information). We sexed, individually marked and measured offspring as soon as they voluntarily detached from the teat. Offspring survival was monitored daily until 80–85 d of age, whereupon families were released at their site of capture. Our estimate of survival to weaning was therefore based on slightly

different criteria in 2004 (alive at ~85 d) and 2003 (recaptured shortly after weaning: ≥ 90 d old).

Statistical analysis. Analyses were run in R 2.3.1 (refs 27, 28) or Genstat 8.0 (ref. 29). The proportion of females with young on every teat was analysed with logistic regression (binomial error) with year (that is rearing environment) and either mating treatment or ejaculate competitiveness as factors. The effect of mating treatment on offspring survival to weaning was analysed as the proportion of a litter that survived (numerator, number alive; denominator, young on teats after birth) using generalized linear mixed models with binomial error (fixed factors: year, mating treatment; random factor: block in 2004). One block was excluded because a male seemed to be infertile and a female died. Separate analyses were run for each year. We also compared offspring survival of monandrous females mated to males of high and low ejaculate competitiveness and polyandrous females using the same modelling approach. We used monthly recapture data to generate Jolly–Seber estimates of post-weaning survival probabilities³⁰.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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