Implications of multiple mating for offspring relatedness and shoaling behaviour in juvenile guppies

Jonathan P. Evans* and Jennifer L. Kelley

Centre for Evolutionary Biology, School of Animal Biology, The University of Western Australia, Nedlands, WA 6009, Australia
*Author for correspondence (jonevan@cyllene.uwa.edu.au).

Polyandry (female multiple mating) can confer important benefits to females, but few studies have considered its potential costs. One such cost may arise through differences in the relatedness of offspring born to females with different mating histories; offspring born to monandrous females are always full siblings, while those produced by polyandrous females may be full or half siblings. These differences may have important consequences for social interactions among offspring. We used artificial insemination in the guppy (*Poecilia reticulata*), a live-bearing freshwater fish with some of the highest reported estimates of polyandry in any vertebrate (Hain & Neff 2007; Neff et al. 2008). Newborn guppies exhibit well-developed shoaling skills (Magurran & Seghers 1990) and are able to discriminate kin from non-kin both through phenotype matching and familiarity (Hain & Neff 2007). In the wild, guppy shoals tend to be size structured and juveniles are thought to remain in their brood groups before dispersing to form adult shoals comprising unrelated individuals (Russell et al. 2004; Hain & Neff 2007). We ask whether the previously documented benefits of polyandry in this species, which include the production of neonates with enhanced shoaling skills (Evans & Magurran 2000), persist when sperm from either two males (polyandry) or a single male (monandry) are artificially inseminated into sexually mature but virgin female guppies. We also determine whether levels of relatedness, which are likely to be more variable in polyandrous broods, influence shoaling behaviour, an important anti-predator trait in this species (Magurran 1990). Grouping behaviour encompasses a time element (e.g. the duration of association) and a spatial component (distance between subjects) (Krause & Ruxton 2002). We consider both of these components as our measures of shoaling behaviour.

1. INTRODUCTION

Polyandry, where females mate with two or more males within a single reproductive cycle, is taxonomically widespread and has profound evolutionary implications for both sexes (Birkhead & Møller 1998). In particular, studies have asked why females should mate multiply when the sperm from just one male are theoretically capable of fertilizing their entire complement of eggs (Keller & Reeve 1995; Yasiu 1997; Jennions & Petrie 2000). While many studies have reported substantial direct and indirect benefits of polyandry (reviewed by Arnvist & Nilsson 2000; Simmons 2005), few have considered its potential costs.

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2008.0423 or via http://journals.royalsociety.org.

2. MATERIAL AND METHODS

Guppies were descendents of wild-caught individuals from the Alligator Creek population in Queensland, Australia. The experimental design involved 25 ‘blocks’ (experimental units) containing two focal males (taken from different tanks within our outbred stock population) and four females (also from different stock aquaria). Within each of these blocks, each pair of males was mated through artificial insemination simultaneously to two of the females (polyandry) and separately with each of the two remaining females (monandry). The absolute number of sperm inseminated was held approximately constant by inseminating the same number of sperm bundles (spermatozoogamata) in both treatments (spermatozoogamata contain approximately equal numbers of sperm, both within individual ejaculates and across different males; Evans et al. 2003). Full methods for artificial insemination are described in detail by Evans & Rutstein (2008).

Broods were isolated from each female following birth for approximately 24 hours, at which time their shoaling behaviour was assessed. Briefly, we estimated the duration that pairs of siblings spent shoaling over a 10 min period and the mean inter-individual distances between each fish using a point-sampling approach. Paternity data for the offspring used in these trials were derived...
from a prior study investigating repeatability in sperm competition success (Evans & Rutstein 2008; see the electronic supplementary materials for full methods).

A total of 66 broods were produced comprising \( n = 553 \) offspring, of which \( n = 520 \) (260 pairs) were tested. Offspring not included \( (n=33) \) came from families with uneven brood sizes, and thus one individual offspring in each of these families was not tested. We used general linear mixed models to investigate both treatment effects (polyandry/monandry) and relatedness (half siblings or full siblings) on offspring traits (electronic supplementary material). Excluding the covariate did not alter the results for relatedness effects on either shoaling \( (p=0.025) \) or mean inter-individual distances \( (p=0.029) \).

Note that this marginal effect of treatment on mean inter-individual distances is influenced by relatedness (GLMM with relatedness added as a random factor: \( F_{1,46.3} = 2.32, p = 0.134 \)).

### 3. RESULTS

Broods were distributed approximately evenly between polyandry \( (n = 35) \); mean number of offspring \( = 8.26 \pm 0.64 \) and monandry treatments \( (n = 31)\); \( 8.52 \pm 0.74 \). Neither brood size nor mean offspring size differed between treatments (GLMM: \( F_{1,45.3} = 0.35, p = 0.85 \); \( F_{1,45.4} = 0.04, p = 0.84 \), respectively). Furthermore, the difference in body length between individual offspring within each pair did not differ between treatments (GLMM: \( F_{1,51.8} = 0.32, p = 0.57 \) or between full- and half-sibling pairs (GLMM: \( F_{1,176.5} = 1.42, p = 0.24 \)).

We obtained relatedness data for 204 offspring pairs \( (n = 174 \) full-sibling pairs and \( n = 30 \) half-sibling pairs). Our analysis revealed no significant effect of mating treatment on shoaling times or inter-individual distances (table 1a). However, we found that levels of relatedness between offspring pairs (half siblings versus full siblings) significantly influenced both of these measures (table 1b). Pairs of full siblings spent a greater proportion of time shoaling (mean \( = 0.74 \pm 0.01, n = 167 \) pairs) than half siblings \( (0.68 \pm 0.03, n = 28 \) pairs; figure 1a). Likewise, mean inter-individual distances were significantly lower in full-sibling \( (53.4 \pm 2.82 \) mm) than in half-sibling pairs \( (68.4 \pm 6.77 \) mm; figure 1b). Not surprisingly, mean inter-individual distances were strongly negatively associated with the proportion of time that pairs of siblings spent shoaling \( (r = 0.71, n = 250, p < 0.0001; \) figure 2).

### 4. DISCUSSION

Our results revealed no overall effect of mating treatment on female fecundity and offspring traits, contrasting with previous work that revealed increases in brood size and both offspring shoaling and predator escape abilities in multiply mated females (Evans & Magurran 2000). However, in Evans & Magurran’s (2000) study, females mated freely with males, thus giving them the opportunity of both pre- and post-copulatory mate choice. Female guppies are known to prefer relatively colourful males (Houde 1997) and these pre-mating preferences are further reinforced through post-copulatory (cryptic) female choice (Pistello et al. 2004). Such preferences would have been undermined by our artificial insemination protocol, thus weakening the strength of sexual selection on male ornamentation.
high- and low-predation populations would be useful to test this idea.

A number of studies reveal that kinship influences shoaling in fish (reviewed by Ward & Hart 2003), but there is surprisingly little evidence that wild shoals are comprised of related individuals. Two microsatellite studies that focused on the levels of relatedness in wild adult guppies reported no evidence for kin structuring (Russell et al. 2004; Hain & Neff 2007). However, Hain & Neff (2007) reported that relatedness among adult pairs was high (16% of pairs were more related than half siblings); such associations may be important for cooperative behaviours such as predator inspection. Nevertheless, in the light of our findings, we advocate further genetic and behavioural studies that include juveniles, since the ability of neonates to recognize and associate with kin may have important fitness implications with respect to predator avoidance, inbreeding and kin selection.

We thank Alison Rutstein for her previous genetic analysis, Rob Brooks for generously providing laboratory space and facilities, Massimiliano Martinelli for access to P BSCI 2.0 software for estimating inter-individual distances, and two anonymous referees for their helpful comments that improved the manuscript. We also thank the Australian Research Council and The University of Western Australia for financial support.


