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

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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, to evaluate shoaling behaviour in polyandrous and monandrous broods. We combined this information with known parentage data for the polyandrous broods to determine whether sibling relatedness influenced offspring shoaling behaviour. While we detected no effect of mating treatment (polyandry/monandry) on shoaling behaviour, we found that pairs of full siblings spent significantly more time shoaling (and in close proximity) than pairs of half siblings. This latter finding confirms the ability of newborn guppies to distinguish brood mates on the basis of kinship, but also suggests an important and hitherto unrealized potential cost of polyandry: a reduction in within-brood relatedness with potentially important implications for offspring social behaviour.

Keywords: kin selection; schooling; good genes; kinship; cryptic female choice; sperm competition

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 & Möller 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.

One obvious implication of polyandry is that a female’s eggs can be fertilized by more than one male, meaning that broods will comprise either paternal half siblings (coefficient of relatedness \( r = 0.25 \)) or full siblings (\( r = 0.5 \)). By contrast, under monandry \( r \) always equals 0.5. The potential effects of such differences in within-brood relatedness under polyandry and monandry for offspring behaviour have rarely been considered. Yet many animals are capable of kin recognition, which in turn can confer a number of advantages (Dugatkin 1997). In salmonid fishes, for example, fry reared in full-sibling groups display less aggression and consequently have higher growth rates than unrelated fish (Brown & Brown 1993), while in sticklebacks individuals that associate with kin are more likely to engage in risky cooperative behaviours such as predator inspection (Milinski 1987).

In this paper, we explore the possible consequences of variability in within-brood relatedness for offspring shoaling behaviour 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.

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 (spermatozeugmata) in both treatments (spermatozeugmata 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 DNA analysis performed by the Centre for Evolutionary Biology, University of Western Australia.
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).

### 3. RESULTS

Broods were distributed approximately evenly between polyandry \( (n = 35) \); mean number of offspring \( \pm \text{s.e.} = 8.26 \pm 0.64 \) and monandry treatments \( (n = 31); 8.52 \pm 0.74 \). Neither brood size nor mean offspring size differed between treatments (GLMM: \( F_{1,64} = 0.35, p = 0.85; F_{1,57.4} = 0.04, p = 0.84, \text{respectively} \)). Furthermore, the difference in body length between individual offspring within each pair did not differ between treatments (GLMM: \( F_{1,52.9} = 0.32, p = 0.57 \) or between full- and half-sibling pairs (GLMM: \( F_{1,178.5} = 1.42, p = 0.24 \)).

We obtained relatedness data for 204 offspring pairs \( (n = 174 \text{ full-sibling pairs and } n = 30 \text{ half-sibling pairs}) \). 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 (full siblings versus half siblings) significantly influenced both of these measures (table 1b). Pairs of full siblings spent a greater proportion of time shoaling (mean \( \pm \text{s.e.} = 0.74 \pm 0.01, n = 167 \text{ pairs} \) than half siblings \( (0.68 \pm 0.03, n = 28 \text{ pairs}; \text{figure 1a}) \). Likewise, mean inter-individual distances were significantly lower in full-sibling \( (53.4 \pm 2.82 \text{ mm}) \) than in half-sibling pairs \( (68.4 \pm 6.77 \text{ mm}; \text{figure 1b}) \). Not surprisingly, mean inter-individual distances were strongly negatively associated with the proportion of time that pairs of siblings spent shoaling \( (r=0.71, n=250, p<0.0001; \text{figure 2}) \).

### 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 (Pilastro et al. 2004). Such preferences have been undermined by our artificial insemination protocol, thus weakening the strength of sexual selection on male ornamentation.

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**Table 1. Effect of mating treatment and relatedness on social interactions in juvenile guppies.**

<table>
<thead>
<tr>
<th>General Linear Mixed Models*</th>
<th>Numerator D.F.</th>
<th>Denominator D.F.</th>
<th>( F )</th>
<th>( p )-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Mating Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoaling Times (Angular Transformed)</td>
<td>1</td>
<td>48.8</td>
<td>0.77</td>
<td>0.385</td>
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<tr>
<td>Inter-individual Distance (Log Transformed)</td>
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<td>48.8</td>
<td>3.69</td>
<td>0.060b</td>
</tr>
<tr>
<td>(b) Relatedness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoaling Times (Angular Transformed)</td>
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<td>181.7</td>
<td>5.25</td>
<td>0.023</td>
</tr>
<tr>
<td>Inter-individual Distance (Log Transformed)</td>
<td>1</td>
<td>182.2</td>
<td>5.18</td>
<td>0.024</td>
</tr>
</tbody>
</table>

*GLMMs showing effects of (a) mating treatment (polyandry/monandry) and (b) relatedness (full siblings/half siblings) on shoaling duration and mean inter-individual distances for offspring pairs. We included differences between offspring size as covariates (all \( p \)-values for covariates >0.138), as this measure was associated with shoaling behaviour (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: \( F_{1,46.5} = 2.32, p=0.134 \)).

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**Figure 1. Shoaling behaviour compared between pairs of full and half siblings. (a) Mean \( \pm \text{s.e.} \) shoaling times and (b) mean \( \pm \text{s.e.} \) inter-individual distances are compared between pairs of full-sibling and half-sibling offspring.**

Our genetic data, coupled with our analysis of offspring behaviour, revealed a significant effect of relatedness on the shoaling behaviour of newborn guppies. This finding confirms the ability of neonates to recognize and associate preferentially with kin according to the level of within-brood relatedness. This effect is unlikely to be due to in utero or post-natal familiarity since offspring were tested within 24 hours of birth and familiarity among shoal mates takes 12 days to develop (post-parturition; Griffiths & Magurran 1997). Another mechanism of recognition which develops over shorter time scales (e.g. 24 hours) is the discrimination of individuals that have experienced a similar odour environment (e.g. similar diets; Ward et al. 2005). However, this is unlikely in the current experiment as post-partum juveniles were kept in their brood groups prior to testing and therefore all brood members experienced the same odour environment. One possibility that warrants further investigation is that offspring use post-natal phenotype matching as a form of kin recognition; this is considered to be more reliable than familiarity in populations that exhibit high levels of multiple mating because individuals are more likely to encounter unfamiliar kin (Hain & Neff 2007).

Our study also reveals a potential fitness cost of polyandry. We found that offspring pairs that were full siblings spent more time shoaling together and in close proximity than half siblings. Since shoaling confers important benefits in terms of predator evasion (Magurran 1990; Pitcher & Parrish 1993), mechanisms that effectively reduce the number of sires contributing to a brood (e.g. female mating decisions and/or the ability to exercise post-copulatory choice) may act to enhance the indirect fitness of females. Consequently, one might expect that the strength of post-copulatory sexual selection, and therefore the average relatedness among brood members, to vary as a function of predation risk, since this will favour increased shoaling (Magurran 1990). The comparison of reproductive skew (i.e. distribution of paternity within multiply mated female broods) among 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.

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