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Cite this article: Fitzpatrick LJ, Gasparini C, Fitzpatrick JL, Evans JP. 2014 Male–female relatedness and patterns of male reproductive investment in guppies. *Biol. Lett.* **10**: 20140166.
<http://dx.doi.org/10.1098/rsbl.2014.0166>

Received: 22 February 2014

Accepted: 12 April 2014

Subject Areas:

behaviour, evolution

Keywords:

cryptic female choice, sperm competition, inbreeding depression, sexual conflict

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Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2014.0166> or via <http://rsbl.royalsocietypublishing.org>.

Male–female relatedness and patterns of male reproductive investment in guppies

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Inbreeding can cause reductions in fitness, driving the evolution of pre- and postcopulatory inbreeding avoidance mechanisms. There is now considerable evidence for such processes in females, but few studies have focused on males, particularly in the context of postcopulatory inbreeding avoidance. Here, we address this topic by exposing male guppies (*Poecilia reticulata*) to either full-sibling or unrelated females and determining whether they adjust investment in courtship and ejaculates. Our results revealed that males reduce their courtship but concomitantly exhibit short-term increases in ejaculate quality when paired with siblings. In conjunction with prior work reporting cryptic female preferences for unrelated sperm, our present findings reveal possible sexually antagonistic counter-adaptations that may offset postcopulatory inbreeding avoidance by females.

1. Introduction

Inbreeding can expose deleterious recessive alleles to selection, thus reducing the fitness of individuals or populations (inbreeding depression [1,2]). Where selection favours inbreeding avoidance, both sexes can adopt mate choice strategies that favour unrelated individuals as mates [3,4]. However, because males and females typically pay different reproductive costs, inbreeding can have asymmetric fitness consequences for both sexes [5–7], thus potentially generating sexually antagonistic responses to inbreeding [8].

In polyandrous species, where females mate with multiple males within a single reproductive episode, females may exploit postcopulatory mechanisms of inbreeding avoidance [e.g. 9] to avoid fertilization by sperm from related males [8,10–12]. In theory, males may also exercise postcopulatory inbreeding avoidance by tailoring the size and/or quality of their ejaculates according to female relatedness [7]. Accordingly, some insect studies have documented male ejaculate tailoring as a response to female relatedness [e.g. 13], although such responses are not universal [14,15].

Here, we examine patterns of male reproductive investment in relation to female relatedness in the guppy (*Poecilia reticulata*), a polyandrous, livebearing poeciliid fish. Guppies are ideally suited for evaluating pre- and postcopulatory inbreeding avoidance by males. Males exhibit flexible mating strategies, such that individuals readily adjust courtship [16] and adopt alternative mating strategies in response to female cues [17]. Furthermore, there is evidence of ejaculate tailoring by males [18,19], and for their part females exercise cryptic female choice against inbreeding by manipulating sperm velocity to favour unrelated mates [20]. Moreover, inbreeding occurs in natural populations [21], and offspring arising from consanguineous matings exhibit impaired survival [22], reductions in courtship by males [23] and declines in both body size and fertility [24]. Thus, we predicted that males would expend less courtship effort and reduce their expenditure on ejaculates when exposed to related females.

Table 1. Effect of male–female relatedness on male sigmoid displays. Results include parameter estimates (\pm s.e.), test statistics (z), and significance (p). Sample size $n = 41$.

source	estimates (\pm s.e.)	z -value	p -value
relatedness treatment	1.86 (\pm 0.82)	2.27	0.02
female standard length (mm)	−0.31 (\pm 0.33)	−0.94	0.35

2. Material and methods

Guppies came from an established pedigree (comprising $n = 23$ families reared through three generations of outbreeding) founded by descendants of wild-caught fish from a feral population in Queensland, Australia. The experimental fish were reared in single-sex tanks until four months old. Two full-sibling males were taken haphazardly from each of the 23 families and assigned to their respective treatment tanks ($35 \times 19 \times 22$ cm) where they were kept for 3 days to acclimatize. After this period (i.e. day 0), sperm were extracted from anaesthetized males to obtain baseline measures of sperm viability, velocity, length and numbers (see the electronic supplementary material).

After the initial sperm assays (day 0), we placed either a full-sibling or an unrelated stimulus female in each male's tank for 40 days. In both treatments, males had visual and olfactory access to the stimulus female housed within a transparent and perforated plastic drinks bottle (12 cm diameter) in the centre of each tank. Each stimulus female was replaced with a full sibling on days 10, 20 and 30 to prevent familiarity from diminishing male sexual interest [25]. We measured the number of sigmoid displays (an S-shaped posture used during courtship) over a 10 min period, as this behaviour is known to predict male reproductive fitness in guppies [26]. After each trial, the female was anaesthetized and measured for body size (standard length, SL).

We performed sperm assays again to evaluate both short- (5 days) and long-term (40 days) adjustments in ejaculate traits. A short-term period of 5 days was chosen to ensure that males had time to replenish their sperm after the initial baseline sperm assays [27]. The long-term period of 40 days encapsulates the guppy's entire spermatogenic cycle [28] and therefore accounts for changes in ejaculate traits that depend on sperm maturation. Repeated sperm extractions do not influence male behaviour or survival [19]. The sperm traits considered in this study are associated with male reproductive fitness in poeciliid fishes [29,30].

We used mixed-effects models to analyse variation in courtship and ejaculate traits. All models included relatedness treatment as a fixed factor and family identity as a random effect. Sigmoid data exhibited a negative binomial error distribution and were analysed using a generalized linear mixed-effects model [31] within the glmmADMB [32] package of R [33]. Female standard length was included as a covariate, as male guppies exhibit preferences for larger females [34,35]. Interactions between covariates and relatedness treatment were not significant. The analysis of ejaculate traits was performed using the lme4 [36] package of R and included the additional fixed effects of time (two levels) and time-by-treatment interactions. Male ID was also included as a random effect to account for repeated sampling of individuals across time periods. Five males had no sperm and one was found to have no intact sperm bundles, so these six individuals were excluded from the analyses ($n = 3$ from each treatment).

3. Results

Males directed significantly more courtship towards unrelated females (table 1 and figure 1). However, males housed

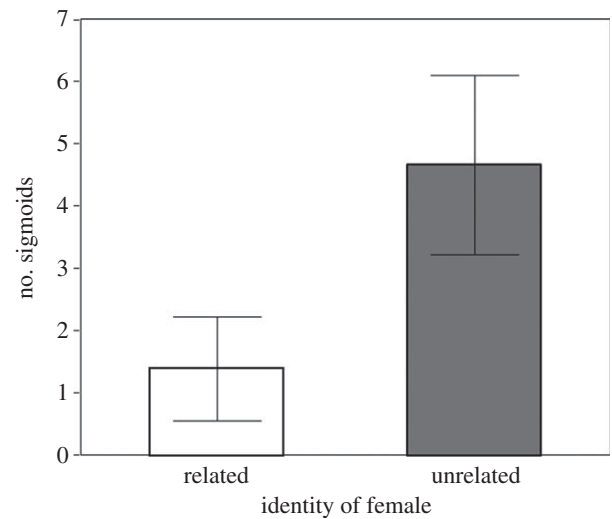


Figure 1. The mean (\pm s.e.) number of courtship displays (sigmoids) by male guppies. White bar indicates related treatment; grey bar indicates unrelated treatment.

Table 2. Effect of male–female relatedness on ejaculate traits in guppies. Test parameters (χ^2) and significance levels (p) are from linear mixed-effects models. Sample size $n = 40$. Sperm viability, proportion live sperm.

source	χ^2	p -value
VAP ($\mu\text{m s}^{-1}$)		
relatedness treatment	4.48	0.03
time	4.51	0.03
treatment \times time	3.67	0.06
sperm viability		
relatedness treatment	0.17	0.68
time	10.74	0.001
treatment \times time	0.70	0.40
sperm length (μm)		
relatedness treatment	0.11	0.73
time	1.83	0.17
treatment \times time	1.64	0.20
sperm count ($\times 10^6$)		
relatedness treatment	0.66	0.42
time	340.17	<0.001
treatment \times time	0.36	0.55

with related females produced sperm with higher average path velocity (VAP) than those housed with unrelated females (table 2). This effect was predominantly attributable to short-term adjustments in VAP (figure 2a), although the treatment-by-time interaction was marginally non-significant (table 2). There was no significant effect of treatment on the remaining ejaculate traits (table 2 and figure 2b–d).

4. Discussion

We show that male guppies are capable of kin recognition and adjust both their courtship and sperm quality accordingly. Males decreased their courtship but produced ejaculates

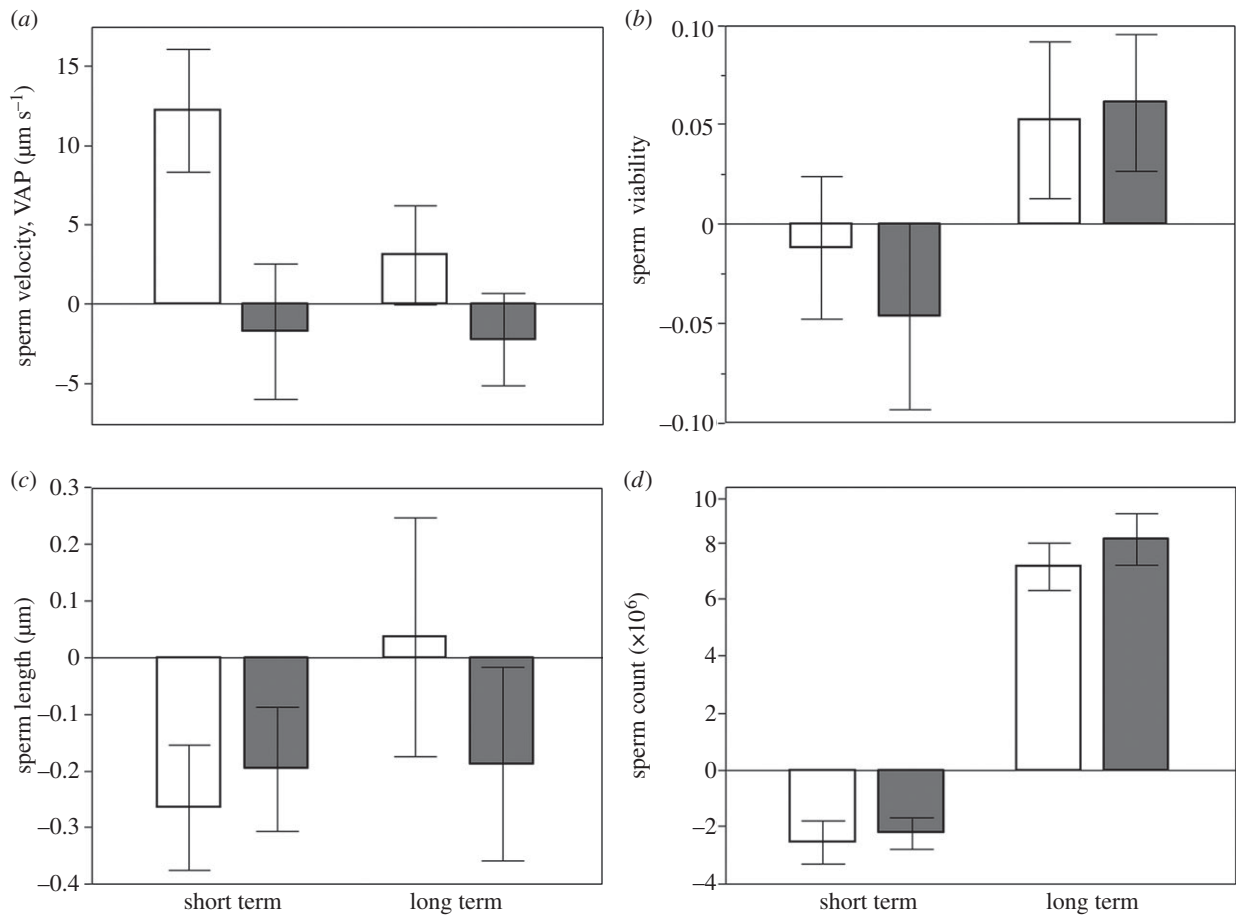


Figure 2. Change in mean (\pm s.e.) sperm velocity (a), viability (proportion live sperm) (b), length (c) and number (d) over short term (difference from baseline to day 5) and long term (difference from baseline to day 40). White bars indicate related treatment; grey bars indicate unrelated treatment.

comprising faster swimming sperm when exposed to siblings. Although this finding contrasts with most other studies, it is not without precedent. In red junglefowl (*Gallus gallus*), males initiate copulations more readily with unrelated females, but when copulations do occur they inseminate more sperm with sisters [8]. Interestingly, as with guppies, female junglefowl exercise cryptic preferences for unrelated male sperm [8], and thus males of both species may adopt sperm investment strategies that counter cryptic female choice against inbreeding.

Our results may reflect sex-specific responses to inbreeding, whereby males tolerate higher levels of inbreeding than females [5–7]. However, while this latter possibility is consistent with our finding for sperm velocity (relatively higher with siblings), it is at odds with our finding that males reduce (precopulatory) mating effect when paired with sisters.

An alternative explanation for our findings is that when females mate with siblings they are more likely to seek additional mates (e.g. where sperm competition/cryptic female choice biases paternity towards less related males

[e.g. 37]). Accordingly, the degree of male–female relatedness may be associated with the level of sperm competition, which in turn can favour a reduction in ejaculate investment under high-‘intensity’ sperm competition scenarios [38]. Thus, our findings for sperm velocity could be interpreted as a response to the heightened intensity of sperm competition when males encounter sibling females.

Finally, although the mechanism underlying kin recognition is unknown in guppies (and in our case may involve familiarity cues developed during pregnancy), other fish species depend on odours linked to genes of the major histocompatibility complex (MHC) to identify kin [e.g. 39,40]. Female guppies have been shown to preferentially associate with particular males based on olfactory cues alone [41], and future work could profitably focus on the MHC as a basis for these odour-based mating preferences.

Acknowledgements. We thank Cameron Duggin for technical assistance and two anonymous reviewers for comments.

References

- Keller LF, Waller DM. 2002 Inbreeding effects in wild populations. *Trends Ecol. Evol.* **17**, 230–241. (doi:10.1016/S0169-5347(02)02489-8)
- Pusey A, Wolf M. 1996 Inbreeding avoidance in animals. *Trends Ecol. Evol.* **11**, 201–206. (doi:10.1016/0169-5347(96)10028-8)
- Hoffman JI, Forcada J, Trathan PN, Amos W. 2007 Female fur seals show active choice for males that are heterozygous and unrelated. *Nature* **445**, 912–914. (doi:10.1038/nature05558)
- Lemaître J-F, Ramm SA, Hurst JL, Stockley P. 2012 Inbreeding avoidance behaviour of male bank voles in relation to social status. *Anim. Behav.* **83**, 453–457. (doi:10.1016/j.anbehav.2011.11.017)
- Kokko H, Ots I. 2006 When not to avoid inbreeding. *Evolution* **60**, 467–475. (doi:10.1111/j.0014-3820.2006.tb01128.x)

6. Parker GA. 1979 Sexual selection and sexual conflict. In *Sexual selection and reproductive competition in insects* (eds MS Blum, NA Blum), pp. 123–166. New York, NY: Academic Press.
7. Parker GA. 2006 Sexual conflict over mating and fertilization: an overview. *Phil. Trans. R. Soc. B* **361**, 235–259. (doi:10.1098/rstb.2005.1785)
8. Pizzari T, Lovlie H, Cornwallis CK. 2004 Sex-specific, counteracting responses to inbreeding in a bird. *Proc. R. Soc. Lond. B* **271**, 2115–2121. (doi:10.1098/rspb.2004.2843)
9. Bretman A, Wedell N, Tregenza T. 2004 Molecular evidence of post-copulatory inbreeding avoidance in the field cricket *Gryllus bimaculatus*. *Proc. R. Soc. Lond. B* **271**, 159–164. (doi:10.1098/rspb.2003.2563)
10. Firman RC, Simmons LW. 2008 Polyandry facilitates postcopulatory inbreeding avoidance in house mice. *Evolution* **62**, 603–611. (doi:10.1111/j.1558-5646.2007.00307.x)
11. Hosken DJ, Blanckenhorn WU. 1999 Female multiple mating, inbreeding avoidance, and fitness: it is not only the magnitude of costs and benefits that counts. *Behav. Ecol.* **10**, 462–464. (doi:10.1093/beheco/10.4.462)
12. Zeh JA, Zeh DW. 1997 The evolution of polyandry II: post-copulatory defenses against genetic incompatibility. *Proc. R. Soc. Lond. B* **264**, 69–75. (doi:10.1098/rspb.1997.0010)
13. Lewis Z, Wedell N. 2009 Male moths reduce sperm investment in relatives. *Anim. Behav.* **77**, 1547–1550. (doi:10.1016/j.anbehav.2009.03.013)
14. Simmons LW, Thomas ML. 2008 No postcopulatory response to inbreeding by male crickets. *Biol. Lett.* **4**, 183–185. (doi:10.1098/rsbl.2007.0578)
15. Stockley P. 1999 Sperm selection and genetic incompatibility: does relatedness of mates affect male success in sperm competition? *Proc. R. Soc. Lond. B* **266**, 1663–1669. (doi:10.1098/rspb.1999.0829)
16. Houde AE. 1997 *Sex, color, and mate choice in guppies*. Princeton, NJ: Princeton University Press.
17. Evans JP, Kelley JL, Ramnarine IW, Pilastro A. 2002 Female behaviour mediates male courtship under predation risk in the guppy. *Behav. Ecol. Sociobiol.* **52**, 496–502. (doi:10.1007/s00265-002-0535-6)
18. Bozynski CC, Liley NR. 2003 The effect of female presence on spermiation, and of male sexual activity on 'ready' sperm in the male guppy. *Anim. Behav.* **65**, 53–58. (doi:10.1006/anbe.2002.2024)
19. Gasparini C, Peretti AV, Pilastro A. 2009 Female presence influences sperm velocity in the guppy. *Biol. Lett.* **5**, 792–794. (doi:10.1098/rsbl.2009.0413)
20. Gasparini C, Pilastro A. 2011 Cryptic female preference for genetically unrelated males is mediated by ovarian fluid in the guppy. *Proc. R. Soc. B* **278**, 2495–2501. (doi:10.1098/rspb.2010.2369)
21. Johnson AM, Chappell G, Price AC, Rodd FH, Olendorf R, Hughes KA. 2010 Inbreeding depression and inbreeding avoidance in a natural population of guppies (*Poecilia reticulata*). *Ethology* **116**, 448–457. (doi:10.1111/j.1439-0310.2010.01763.x)
22. Nakadate M, Shikano T, Taniguchi N. 2003 Inbreeding depression and heterosis in various quantitative traits of the guppy, *Poecilia reticulata*. *Aquaculture* **220**, 219–226. (doi:10.1016/S0044-8486(02)00432-5)
23. Mariette M, Kelley JL, Brooks R, Evans JP. 2006 The effects of inbreeding on male courtship behaviour and coloration in guppies. *Ethology* **112**, 807–814. (doi:10.1111/j.1439-0310.2006.01236.x)
24. van Oosterhout C, Smith AM, Hanfling B, Ramnarine IW, Mohammed RS, Cable J. 2007 The guppy as a conservation model: implications of parasitism and inbreeding for reintroduction success. *Conserv. Biol.* **21**, 1573–1583.
25. Kelley JL, Graves JA, Magurran AE. 1999 Familiarity breeds contempt in guppies. *Nature* **401**, 661–662. (doi:10.1038/44314)
26. Evans JP, Magurran AE. 2001 Patterns of sperm precedence and predictors of paternity in the Trinidadian guppy. *Proc. R. Soc. Lond. B* **268**, 719–724. (doi:10.1098/rspb.2000.1577)
27. Kuckuck C, Greven H. 1997 Notes on the mechanically stimulated discharge of spermiozeugmata in the guppy, *Poecilia reticulata*: a quantitative approach. *Z. Fischkunde* **4**, 73–88.
28. Billard R, Puissant C. 1969 La spermatogenèse de *Poecilia reticulata* II: la production spermatogénétique. *Ann. Biol. Anim. Bioch. Biophys.* **9**, 307–313. (doi:10.1051/md:19690301)
29. Boschetto C, Gasparini C, Pilastro A. 2011 Sperm number and velocity affect sperm competition success in the guppy (*Poecilia reticulata*). *Behav. Ecol. Sociobiol.* **65**, 813–821. (doi:10.1007/s00265-010-1085-y)
30. Smith CC. 2012 Opposing effects of sperm viability and velocity on the outcome of sperm competition. *Behav. Ecol.* **23**, 820–826. (doi:10.1093/beheco/ars036)
31. Faraway JJ. 2005 *Extending the linear model with R: generalized linear, mixed effects and nonparametric regression models*. CRC Texts in Statistical Science. London, UK: Chapman & Hall.
32. Fournier DA, Skaug HJ, Ancheta J, Ianelli J, Magnusson A, Maunder M, Nielsen A, Sibert J. 2012 AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optim. Methods Softw.* **27**, 233–249. (doi:10.1080/10556788.2011.597854)
33. R Development Core Team. 2013 *A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing (<http://www.R-project.org/>).
34. Dosen LD, Montgomerie R. 2004 Female size influences mate preferences of male guppies. *Ethology* **110**, 245–255. (doi:10.1111/j.1439-0310.2004.00965.x)
35. Herdman EJE, Kelly CD, Godin JGJ. 2004 Male mate choice in the guppy (*Poecilia reticulata*): do males prefer larger females as mates? *Ethology* **110**, 97–111. (doi:10.1111/j.1439-0310.2003.00960.x)
36. Bates D, Maechler M, Bolker BM, Walker S. 2014 Linear mixed-effects models using Eigen and S4. (<http://cran.r-project.org/web/packages/lme4/index.html>)
37. Harano T, Katsuki M. 2012 Female seed beetles, *Callosobruchus chinensis*, remate more readily after mating with relatives. *Anim. Behav.* **83**, 1007–1010. (doi:10.1016/j.anbehav.2012.01.022)
38. Parker GA, Ball MA, Stockley P, Gage MJG. 1996 Sperm competition games: individual assessment of sperm competition intensity by group spawners. *Proc. R. Soc. Lond. B* **263**, 1291–1297. (doi:10.1098/rspb.1996.0189)
39. Olsén KH, Grahn M, Lohm J, Langefors Å. 1998 MHC and kin discrimination in juvenile Arctic charr, *Salvelinus alpinus* (L.). *Anim. Behav.* **56**, 319–327. (doi:10.1006/anbe.1998.0837)
40. Reusch TBH, Haberli MA, Aeschlimann PB, Milinski M. 2001 Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature* **414**, 300–302. (doi:10.1038/35104547)
41. Shohet AJ, Watt PJ. 2004 Female association preferences based on olfactory cues in the guppy, *Poecilia reticulata*. *Behav. Ecol. Sociobiol.* **55**, 363–369. (doi:10.1007/s00265-003-0722-0)