



The ontogeny of courtship, colour and sperm production in male guppies

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The onset of sexual behaviour in three age classes of male guppy *Poecilia reticulata* occurred in synchrony with the appearance of body colouration and anal fin differentiation but not with sperm production. There was also a positive correlation between sperm production and sexual behaviour in the two older age classes.

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The reproductive biology and behaviour of guppies *Poecilia reticulata* (Peters) has been extensively described (Baerends *et al.*, 1955; Liley, 1966; Constantz, 1989; Houde, 1997). Like other poeciliid fishes, guppies are livebearers with internal fertilization in which the male transfers sperm to females via a modified anal fin known as a gonopodium. Male courtship consists of an elaborate display in which the male orientates himself in front of the female and performs an S-shaped posture known as a 'sigmoid display' (Liley, 1966). In general, females are attracted to large-bodied, colourful males with high courtship display rates (Houde, 1997). In addition to solicited copulations, males can use the alternative method of gonopodial thrusting to achieve insemination (Liley, 1966; Luyten & Liley, 1991). During gonopodial thrusts the male approaches the female from behind, swings his gonopodium forward, and attempts to insert it into her genital opening without her co-operation.

Although Houde (1997) briefly describes the development of male reproductive traits in her recent monograph, little is known about the ontogeny of courtship behaviour in relation to other sexual traits such as body colouration and sperm production. Reznick & Bryga (1996) and Reznick *et al.* (1997) showed that wild males typically mature at *c.* 50 days (high-predation sites) or 60 days (low-predation localities) after birth. These figures are based on the maturation of the gonopodium. Males are considered sexually mature when the apical hood of the gonopodium extends beyond the tip of the anal fin, usually at *c.* 7 weeks of age (Houde, 1997). It is not known, however, whether adult patterns of sperm production can be achieved in such young fish. Furthermore, it is not clear whether courtship behaviour, morphology (including colour patterns) and spermatogenesis mature in synchrony in male guppies. The ontogeny of these traits were therefore examined in three age classes of male descended from a high-predation population in Trinidad.

The guppies used in this experiment were second generation descendants of wild-caught fish from the lower Tacarigua River, Trinidad. To obtain males of known age, 60 females were isolated and checked daily for broods. All offspring born within designated 2 week periods were isolated from their mother and randomly placed among several

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aquaria (30 × 24 × 20 cm containing an airstone, gravel and weed) where they were fed a diet of Tetramin[®] baby food, *Artemia* nauplii and subsequently commercially prepared flake food. Approximately 300 juveniles were collected during this period and on average each tank accommodated *c.* 40–50 fish. Temperature was maintained between 24.5 and 25.5°C and illumination provided by an 18 W bulb on a 12 L : 12 D cycle.

At *c.* 5 weeks of age males became morphologically distinguishable from females on the basis of anal fin development and emerging colour patterns. By 7–10 weeks of age, several males ($n=18$) had developed rod-shaped gonopodia with clearly visible apical hoods (Houde, 1997). These males (hereafter termed 'juveniles'), which were taken randomly from several tanks, were placed in groups of five in an observation tank (59 × 29 × 35 cm) containing five adult, non-virgin females and allowed to settle overnight. The females used during the courtship trials were taken from stock aquaria and not from the tanks containing the experimental fish. All females were therefore unfamiliar (and unrelated) to the test males. On the following morning the number of sigmoid displays and gonopodial thrusts performed by each male over a 15 min period were recorded. This procedure was repeated for males aged 13 weeks ($n=50$) and 26 weeks ($n=50$; hereafter referred to as 3 and 6 month olds respectively).

Following behavioural observations males were isolated for 2 days to replenish sperm reserves prior to stripping (Kuckuck & Greven, 1997; Pilastro & Bisazza 1999) and anaesthetized in a water bath containing 0.4 g l⁻¹ benzocaine (ethyl p-amino benzoate). After removing excess water from the body surface, standard length (L_S) was recorded for each fish using a dissecting microscope fitted with a digital graticule. All measurements were accurate to within 0.1 mm. Each male was then photographed using a mounted camera (Nikon FM 35 mm with a 100 mm macro lens and two electronic flash guns) loaded with Kodak Ektachrome 64 slide film. For calibration, a section of ruler was included in each photograph. Slides were digitized and colours were quantified using a computerized image analysis software package (NIH image). The total surface area of each fish was measured and the percentage of total area covered by two main colour types: carotenoids (which include red, orange and yellow) and black spots and lines (melanin) (Endler, 1978) was calculated. As relative area of colouration was used, the possible confounding effect of body size differences among the age classes was eliminated.

Established techniques were used (Kuckuck & Greven, 1997; Matthews *et al.*, 1997) to strip sperm from anaesthetized males. Briefly, the gonopodium was swung forward and gentle pressure applied to the side of the abdomen, just anterior to the base of the gonopodium. This action released a number of spermatozeugmata (sperm bundles). After breaking down sperm bundles and checking for an even distribution of sperm cells within each sample (Matthews *et al.*, 1997), sperm counts were estimated by counting sperm cells on an 'improved Neubauer chamber' haemocytometer under × 400 magnification. Counts were expressed as the total number of spermatozoa per stripped ejaculate.

Of the 18 juvenile males tested, only one produced sperm when stripped. Despite this, 12 of these males performed sexual behaviour, albeit at a lower rate than their adult counterparts [Fig. 1(a), (b)]. After controlling for the potentially confounding affect of body size on male behaviour 6 month old males performed significantly more sigmoid displays (ANCOVA with L_S as the covariate: $F_{2,110}=7.16$, $P<0.0001$) and gonopodial thrusts ($F_{2,110}=11.78$, $P<0.0001$) than those in the other age classes [Fig. 1(a), (b)]. Sperm number showed a corresponding pattern to sexual behaviour in adult males; 6 month old males produced significantly higher numbers of sperm (controlling for L_S) than the other age classes [ANCOVA, $F_{2,110}=12.09$, $P<0.0001$; Fig. 1(c)]. Furthermore, 6 month old males had a significantly greater percentage of their body covered with carotenoid pigmentation (Kruskal–Wallis, $H_{2,95}=16.46$, $P<0.001$) and black spots ($H_{2,96}=47.94$, $P<0.001$) than younger fish [Fig. 1(d)]. L_S did not significantly differ among the age classes (ANOVA, $F_{2,110}=0.79$, $P=0.45$).

There was a highly significant correlation between sigmoid display rate and the number of sperm per stripped ejaculate in 3 month (Pearson's correlation coefficient, $r=0.85$, $n=50$, $P<0.0001$) and 6 month old fish ($r=0.73$, $n=50$, $P<0.0001$). The correlations between sigmoid display rate and the number of sperm in both groups remained

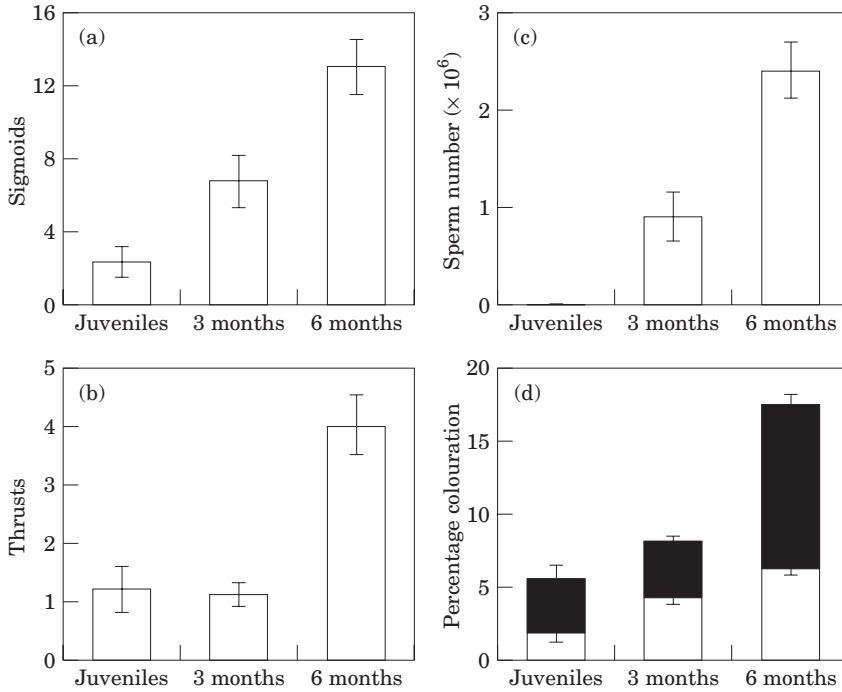


FIG. 1. Mean (\pm S.E.) number of (a) sigmoid displays and (b) gonopodial thrusts over a 15 min period in three age classes of male guppy; (c) mean (\pm S.E.) number of sperm per stripped ejaculate and (d) percentage colouration (■, % melanin, □, % carotenoids) in three age classes of male.

significant after applying the Dunn-Šidák adjustment ($\alpha=0.05$ corrected to $\alpha'=0.017$) for multiple comparisons (Sokal & Rohlf, 1981). A similar pattern was evident for gonopodial thrusts; thrust rate was found to be correlated with the number of stripped sperm in both 3 month ($r=0.43$, $n=50$, $P=0.002$) and 6 month old fish ($r=0.35$, $n=50$, $P=0.014$). Sperm number was also found to be positively correlated with L_S in 3 month ($r=0.44$, $P=0.001$) and 6 month old fish ($r=0.43$, $P=0.002$).

As described by Houde (1997), males begin to show sexual behaviour as the gonopodium differentiates, usually at *c.* 7 weeks. Specifically, male guppies are not considered sexually mature until the apical hood, a sensory protuberance on the gonopodium, extends beyond the tip of the gonopodium. In the present study, the relative length of the apical hood in the juvenile age class was not specifically examined. The courtship behaviour of these fish, however, was only recorded if their gonopodia were indistinguishable (by eye) from other adult males, ignoring those males that were obviously incapable of inseminating females. Despite this, all but one of the males in the juvenile group did not produce sperm when manually stripped. Thus, the data indicate that male guppies begin to court females before they are capable of fertilizing their eggs. Similar results were presented by Bisazza *et al.* (1996) when they studied male sexual behaviour in another poeciliid, the eastern mosquitofish *Gambusia holbrooki* Girard. In that study they found that the sexual activity of immature male eastern mosquitofish was as high as that of adults despite the fact that they were unable to transfer sperm. Because the reproductive success of male eastern mosquitofish is strongly dependent on body size, which in turn is influenced by adult sex ratio, Bisazza *et al.* (1996) suggested that male eastern mosquitofish used sexual behaviour to ‘predict’ their future reproductive success at a given size. Alternatively, effective courtship behaviour in physiologically mature males might depend on early experience. Investigations of antipredator behaviour reveal that early experience plays an important role in development (Huntingford & Wright,

1993). It thus remains to be tested whether the precocious behaviour of young male guppies is adaptive or simply a by-product of physiological development.

The present study revealed that the rate at which males performed sigmoid displays, gonopodial thrusts and produced sperm was greatest in 6 months old fish. The extent of carotenoid and melanistic colouration was also highest in these fish corresponding with maximum levels of sperm production and courtship activity. A significant correlation was detected between sperm number and male sexual behaviour in both age classes of adult male which is consistent with the results presented by Matthews *et al.* (1997) when they tested Sheldon's (1994) fertility advertisement hypothesis. Although the present study was not specifically designed to test this hypothesis further, the data suggest that courtship rate is indeed a reliable indicator of the number of sperm over the reproductive lifespan of males. Additionally, it was found that L_S correlated with sperm number (Pilastro & Bisazza, 1999). Reynolds & Gross (1992) showed that female guppies were attracted to large males, and that male size had significant father-son heritability. Large-bodied males with high courtship rates may therefore have a twofold advantage over their smaller, less vigorous counterparts because they are likely to be more successful during both pre-copulatory (female choice) and post-copulatory (sperm competition) sexual selection.

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References

- Baerends, G. P., Brouwer, R. & Waterbolk, H. T. (1955). Ethological studies on *Lebistes reticulatus* (Peters), I. An analysis of the male courtship pattern. *Behaviour* **58**, 669–675.
- Bisazza, A., Pilastro, A., Palazzi, R. & Marin, G. (1996). Sexual behaviour of immature male eastern mosquitofish: a way to measure intensity of intra-sexual selection? *Journal of Fish Biology* **48**, 726–737.
- Constantz, G. D. (1989). Reproductive biology of poeciliid fishes. In *Ecology of Livebearing Fishes* (Meffe, G. K. & Snelson, F. F., Jr, eds), pp. 33–50. New Jersey: Prentice-Hall.
- Endler, J. A. (1978). A predator's view of animal color patterns. *Evolutionary Biology* **11**, 319–364.
- Houde, A. E. (1997). *Sex, Color, and Mate Choice in Guppies*. New Jersey: Princeton University Press.
- Huntingford, F. A. & Wright, P. J. (1993). The development of adaptive variation in predator avoidance in freshwater fishes. In *Behavioural Ecology of Fishes* (Huntingford, F. A. & Torricelli, P., eds), pp. 45–61. Chur, Switzerland: Harwood Academic Publishers.
- Kuckuck, C. & Greven, H. (1997). Notes on the mechanically stimulated discharge of spermiozeugmata in the guppy, *Poecilia reticulata*: A quantitative approach. *Zeitschrift für Fischkunde* **4**, 73–88.
- Liley, N. R. (1966). Ethological isolating mechanisms in four sympatric species of poeciliid fishes. *Behaviour* (Suppl. XIII) **13**, 1–197.
- Luyten, P. H. & Liley, N. R. (1991). Sexual selection and competitive mating success of male guppies (*Poecilia reticulata*) from four Trinidad populations. *Behavioural Ecology and Sociobiology* **28**, 329–336.
- Matthews, I. M., Evans, J. P. & Magurran, A. E. (1997). Male display rate reveals ejaculate characteristics in the Trinidadian guppy *Poecilia reticulata*. *Proceedings of the Royal Society of London B* **264**, 695–700.
- Pilastro, A. & Bisazza, A. (1999). Insemination efficiency of two alternative male mating tactics in the guppy (*Poecilia reticulata*). *Proceedings of the Royal Society of London B* **266**, 1887–1891.
- Reynolds, J. D. & Gross, M. R. (1992). Female mate preference enhances offspring growth and reproduction in a fish, *Poecilia reticulata*. *Proceedings of the Royal Society of London B* **264**, 57–62.
- Reznick, D. N. & Bryga, H. A. (1996). Life-history evolution in guppies (*Poecilia reticulata*: Poeciliidae). 5. Genetic basis of parallelism in life histories. *The American Naturalist* **147**, 339–359.
- Reznick, D. N., Shaw, F. H., Rodd, F. H. & Shaw, R. G. (1997). Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science* **275**, 1934–1937.
- Sheldon, B. C. (1994). Male phenotype, fertility, and the pursuit of extra-pair copulations by female birds. *Proceedings of the Royal Society of London B* **257**, 25–30.
- Sokal, R. R. & Rohlf, F. J. (1981). *Biometry*. San Francisco: Freeman.