Parasitized female guppies do not prefer showy males

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In many species male sexual characteristics are known to be affected negatively by parasites, which render their hosts unattractive to females, but how parasites affect the mating decisions of their female hosts has received little attention. The monogenean parasite Gyrodactylus turnbulli reduces the sexual display and colour intensity of male guppies, Poecilia reticulata, which makes them less attractive to females. Here, I examine how these parasites affect the mate choice behaviour of their female hosts. Virgin females were experimentally exposed to G. turnbulli and allowed to choose between an attractive and an unattractive male in a simultaneous choice test. Infected females were significantly less discriminatory than healthy ones and their level of activity during choice trials was reduced with increasing parasite load, suggesting an energetic constraint imposed by the parasites. This result implies that sexual selection pressure for male showiness is diminished, which is consistent with recent theoretical models.

Since Hamilton & Zuk (1982) published their handicap hypothesis, which suggests that females prefer showy males because showiness reveals resistance to parasites, much effort has been put into investigating the effect of parasites on male ornaments (e.g. Kennedy et al. 1987; Milinski & Bakker 1990; Møller 1990; Zuk et al. 1990; Burley et al. 1991; Johnson & Boyce 1991; Houde & Torio 1992; López 1998a, b). However, the question of how parasitism affects female mate choice for such ornamented males has received very little attention (Poulin 1994; Poulin & Vickery 1996).

Parasites could alter a female’s choosiness in two ways: (1) by directly affecting her decision making; or (2) by weakening the female and thereby imposing an energetic cost on her. The first mechanism could evolve if the parasite’s interests are different from the host’s (Milinski 1990), for example if its female host does not choose the showiest males. If the second mechanism occurs, the parasitized female suffers an energy loss and will therefore be less likely to engage in costly mate assessment behaviour or in resisting unwanted males, than a healthy female. In a theoretical analysis, Real (1990) predicted that, when the costs of mate sampling and choosing are increased, a female should sample less and be less choosy. Milinski & Bakker (1992) tested this prediction on sticklebacks, Gasterosteus aculeatus, and found that with increasing time and energy costs of moving between males (females had to swim against a current), previously unattractive males became highly acceptable. Similarly, a weakening effect of parasites could lead to reduced mate selectivity in infected females.

Whether the effect of parasites on female choice is explained via the first or second mechanism, or both, the consequences for the handicap theory remain the same: recent theoretical models predict that such an effect would diminish sexual selection pressure on male showiness (Poulin & Vickery 1996). In the present study I examined the effect of Gyrodactylus turnbulli, a common parasite of guppies, Poecilia reticulata, on female mate choice behaviour.

Guppies are polygamous live-bearers with internal fertilization and breed throughout the year. Male guppies from the Paria River in Trinidad have large orange spots. Females from this population base their mate choice on the intensity of the orange colour and on the sigmoid display rates of males (Kennedy et al. 1987; Houde & Torio 1992; López 1998a). Males can adopt two alternative strategies to achieve copulations: they can try to persuade females to mate with them by courting in front of them, vibrating and bending their bodies in an arch shape (termed sigmoid displays), or they can try to sneak a copulation (Farr 1989; Magurran & Seghers 1990; Godin 1995). Females invariably try to resist sneaky males by fleeing from them. Males often follow such fleeing females obstinately, attempting to force a copulation. Displaying males are mostly ignored, but sometimes accepted by receptive females. When a female accepts a particular male, she smoothly glides towards him. Male and female then start swimming rapidly together in circles, until the male moves behind and below her to insert his gonopodium and transfer sperm (e.g. Liley...
Both female behaviour patterns, resisting males and cooperating with them, are likely to be costly in terms of energy.

In the Paria River *G. turnbulli* is a very abundant and probably the only prevalent macroparasite of guppies (Lyles 1990; personal observation). It is an external monogenean parasite that hooks itself on to the host, preferentially on the fins. It reduces sexual display rates in male hosts (Kennedy et al. 1987) as well as the orange intensity of the carotenoid spots, consequently leaving its hosts less attractive to females (Houde & Torio 1992). The parasite is present on males and females in the wild (mean infection intensity: 3.9 parasites per host; mean prevalence: 54.5%; Lyles 1990), but no attempt has been made to trace its effect on female characteristics.

In this study, virgin females were experimentally exposed to *G. turnbulli* and then allowed to choose between an attractive and an unattractive male. I compared their mate preference and their level of activity during mate choice with those of control females.

### METHODS

#### Study Animals

I used 46 virgin females of similar age (5–6 months), first-generation descendants from wild guppies caught as juveniles at the Paria River in Trinidad. Females were reared in 40-litre tanks, at a density of 12–14 fish/tank and were fed daily on Tetramin flakes. Day cycles were set at 12:12 h dark:light and the water temperature was 24.5°C. All females had the same father, but different mothers. I selected two adult males from an all-male stock tank, containing 14 individuals. Stocking conditions for males were the same as for females and none of the fish I used had been exposed to *G. turnbulli* previously. Aiming to pick one showy (attractive) male and one less showy (less attractive) male, my first selection criterion was ‘individual display rate’ and my second was ‘orange brightness’. I counted male displays over 6 min for each individual within their stocking tank. Then the two males with the highest display rates (i.e. 28 and 40 per 6 min) and the two males with the lowest display rates (both zero) were placed singly into transparent bottles. Five persons were asked independently to rank the selected males according to the intensity of their orange colour. Rank points were given from 0 to 3. The lowest ranked male received 0 rank points for orange intensity and his display rate was also 0. The highest ranked male received 11 rank points and his display rate was 28. Thus, the lowest displaying, dullest male and the highest displaying, brightest male were selected for the experiment. I chose this two-male-only design since it allowed me to use a relatively small sample size of females, which was limited to 46 individuals.

#### Infection and Disinfection

Thirty females were infected with three to four flukes and 16 females were sham-infected and served as controls. The females were infected (or sham infected) in a randomized order on 10 consecutive days (maximum five females per day), such that each female could enter the subsequent experiment in the same order with a 7-day-old infection (see next section). During the 7 days, the parasites reproduced on the fish, such that females ended up with different numbers of flukes at the time of the experiment (see Results).

Females to be infected were placed individually on a small petri dish (diameter 3 cm) with just enough water to cover the fish when the dish was inclined. In this position the fish slowed down after a while, which allowed me to put a small piece of infected fin from a donor next to the fish, while observing it with a dissecting microscope. I removed the donor fin after three to four parasites had passed over to the recipient fish, which typically occurred within a few seconds to 7 min. I sham infected control fish by holding them the same way in the petri dishes except that they were not exposed to parasites. I obtained the donor pieces by killing (by a cut through the neck) one heavily infected guppy several hours before the experimental infection. This guppy came from a tank of fish that were naturally infected.

All fish were treated with the antiparasitic medication ‘Life Bearer’ (0.0-dimethyl 1-hydroxy 2-trichloromethyl phosphate, Aquarium products) to remove parasites, after the observation trials described below.

#### Observation Trials

All observation trials were conducted in the same experimental tank (40 litres, 45 × 30 × 30 cm). The tank contained an air stone in the centre and a small (18 × 12 × 12 cm) transparent inset tank at each end. These small inset tanks were completely submerged in the water of the experimental tank. They contained a small moss plant, and were covered with perforated plastic lids, which were held in place by stones. The experimental tank was divided in half (each half containing a small inset tank) by a line drawn on the glass.

I made observations in the mornings between 0800 and 1230 hours. Each of the previously selected males (one showy, one less showy) was placed in one of the inset tanks and given 20 min to habituate. Then a female was introduced into the experimental tank (in the randomized order described above) and allowed to habituate for 10 min before observation began. I observed each female twice for 10 min/observation in the same tank, but with the male positions exchanged. Each day up to five females were tested. Male positions were switched once per day only, after one set of females (maximum 5) had been observed. In this new position, males were given 20 min to habituate before I observed the females again. For the data analysis, counts of a measured variable were averaged over both observations. I recorded the following variables: ‘sigmoid displays’ performed by each male; the number of ‘female glides’ towards each male; and the number of tank-side changes a female made. The latter served as a measure for the level of activity of a female. I judged that a female was gliding towards a male if she ceased her current activity, oriented towards a male and...
moved unambiguously in a slow, gliding motion towards him (Houde 1987, 1988). The number of female glides towards a male is an accurate measure of female preference, since it often results in cooperative copulation and affects male mating success positively (Houde 1988). However, in the present experimental set-up it was possible that males could discriminate between females by courting more in front of uninfected ones. To control for this possible effect, I further calculated an index for female preference: the fraction of female gliding responses per male sigmoid display.

After the observation trials, I scanned females for parasites under a dissecting microscope, and recorded Gyrodactylus numbers. All females were then disinfected as described above.

**Statistical Analyses**

Nonparametric tests were used for all analyses. For comparisons between female groups I performed Kruskal–Wallis one-way ANOVAs. For post hoc comparisons within a female group (e.g. glides towards showy male versus glides towards less showy male for infected females only) Kolmogorov–Smirnov paired sample tests were used.

Additionally, Spearman rank correlations were determined to test for the relationship between fluke numbers on females and (1) their swimming activity, (2) their total number of glides and (3) their difference in mate preference.

All $P$ values represent two-sided probabilities.

**Ethical Note**

To establish the level of infection required (moderate), I conducted a pilot experiment using lower levels of infection first but I found that these did not establish well. There was no mortality during or after the infection procedure. All the infected females I used looked healthy, that is, did not look different from uninfected females to the human eye. If a female had shown any sign of distress (e.g. jerky swimming, scratching), which none of them did, I would have treated her immediately to remove parasites and she would have been excluded from the experiment.

**RESULTS**

The difference in mate preference (preference for showy male minus preference for less showy male) is significantly different between control females and infected females (Kruskal–Wallis test: glides towards male: $U=407.5, N_1=16, N_2=30, P<0.001$; glides per sigmoid display: $U=345.0, N_1=16, N_2=30, P=0.014$; Fig. 1). Figure 1 shows that this significant difference between groups is probably generated by control females expressing a marked preference for the showy male while infected females showed no particular preference. This is confirmed by post hoc comparisons (Kolmogorov–Smirnov paired sample tests: control females: glides towards male: $D=0.625, P=0.002$; glides per sigmoid display: $D=0.438, P=0.06$; infected females: glides towards male: $D=0.233, P=0.334$; glides per sigmoid display: $D=0.2, P=0.522$).

Both males performed more sigmoid displays to control females than infected females ($U=343.0, N_1=16, N_2=30, P=0.017$; Fig. 2), and the showy male displayed significantly more than the less showy male in front of both control and infected females (Kolmogorov–Smirnov tests: $D=0.500, P=0.023$ and $D=0.334, P=0.043$ respectively; Fig. 2). Control females swam more frequently between males than infected females ($U=413.0, N_1=16, N_2=30, P<0.001$; Fig. 3), and also performed more glides towards males ($U=362.5, N_1=16, N_2=30, P=0.005$; Fig. 1a). Furthermore, there is a negative correlation between the number of parasites that infected females were carrying after 7 days of infection and (1) their level of activity ($r=-0.829$, $P<0.001$).

Figure 1. Preference of female guppies for the showy male (■) and the less showy male (□) in a two-male choice test measured as (a) number of glides towards a male and (b) the proportion of female glides per male sigmoid display. $N=16$ controls, 30 females infected with Gyrodactylus turnbulli. Means±SE are indicated.
Infected females showed significantly less discriminatory mate preferences than healthy females. This effect appeared for both measurements of preference used: glides towards a male and glides per male sigmoid display.

The latter measure of female preference controls for a possible discriminating effect of males. A male may discriminate against infected females to avoid getting...
infected himself. This could then cause discriminated females to respond less to them. The males in the experiment did display less to infected females than to control females and it was the showy male who mainly reduced his display in front of infected females (Fig. 2), which raises the possibility that he might have been discriminating against them. However, infected females still received significantly more courtship from the showy male than from the less showy one. This suggests that females should be able to discriminate between the males (which they did not), and that the showy male was probably not trying to avoid infected females, or if he was, he did not succeed. An alternative, perhaps more plausible, explanation for reduced courtship in front of infected females is that it results from a reduced sexual response by these females. Such an effect may particularly be manifested here because virgin females were used. Virgin females are highly sexually responsive to males and they may glide towards a male that is not showing courtship, a behaviour that I saw during this experiment.

In addition to showing no preference for attractive males, infected females were generally less active and glided less than healthy ones. This result suggests that they are suffering an energetic cost. More evidence that infected females are energetically constrained is the strong negative relationships between parasite numbers on a female and both her level of activity and her total gliding activity.

What are the consequences of such a parasitic effect for females in a natural environment? Guppy females are usually courted or sneaked up on by several males at the same time (Magurran & Seghers 1994). A choosy female has to be alert, actively resist sneaky males and cooperate in copulation circling when she chooses a particular male. Resisting and cooperating (as described before) are likely to be energetically costly, and weakened, parasitized females would probably not be able to meet these costs. Under these circumstances sneaky males may increase their mating success and displaying males may get fewer mating opportunities. The implications of this parasitic effect for the handicap hypothesis (Hamilton & Zuk 1982) are considerable. Gyrodactylus turnbulli appears to be equally abundant on males and females in the wild (Lyles 1990), and male showiness is known to be negatively correlated with parasite burden (Kennedy et al. 1987; Houde & Torio 1992) as well as positively associated with resistance to these parasites (López 1998a). Since infected females do not prefer attractive, probably resistant, males and may even allow more sneaky copulations by unattractive, probably less resistant, males, sexual selection pressure for male showiness is likely to be diminished as predicted by theory (Poulin & Vickery 1996). To my knowledge, this is the first study reporting a reduced preference by female hosts for showy males whose sexual characters may reveal their ability to cope with parasites, although a very similar parasitic effect on female mate choice was found in a fish species where parasites probably do not affect the male trait over which females exercised choice, that is, male size (Poulin 1994).

From the present data it remains unclear whether this effect on female choice was caused by a direct or indirect parasitic manipulation of females. Although the results point towards an indirect parasitic effect, where females are probably weakened by infection and energetically constrained in their mate choice behaviour, a direct parasitic manipulation cannot be ruled out. For a direct manipulation to occur, the parasite’s interests must be different from its host’s (Miliński 1990). A guppy female benefits by mating with selected showy males which are less likely to harbour infections and may be resistant to G. turnbulli (Kennedy et al. 1987; Houde & Torio 1992; López 1998a), whereas the parasite needs to get its host in physical contact with many other potential hosts to ensure transmission (Bakke et al. 1992; López 1998b). Therefore a direct manipulation should result in infected females coming into contact with more conspecifics than uninfected ones, which is exactly what may happen when females are weakened and not able to resist sneaky males.

The reported reduction in female preference for showy males may affect parasite transmission success in different ways. If infected females do come into contact with many sneaky males, then parasite transmission and reproduction may be enhanced through frequent contacts with a whole spectrum of unattractive, probably nonresistant, males (López 1998a, b). Alternatively, if infected females come into contact with generally fewer mates (e.g. because they are avoided by males), transmission to other hosts may be more difficult and the parasites may be put under higher selection pressure. Future experiments on this host–parasite system should concentrate on the direct interaction of female hosts with a wide range of potential mates to obtain more information about the consequences of this parasitic effect on G. turnbulli transmission and on female mate selection.

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References


