Acquired resistance affects male sexual display and female choice in guppies

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Is resistance to parasites related to the expression of male secondary sex characters? Handicap models predict a positive relationship, proposing that males displaying extravagant sex characters may be honestly signalling their resistance to females. However, no current evidence addresses whether individual changes in immunity (acquired resistance) are reflected in sexual traits. In this experiment I use guppies to compare male orange colour, sigmoid display and female preferences for individual males, before and after a primary challenge infection of males. Challenge infections were terminated chemically and fish were given ten days' recovery time before proceeding with the second measurements. The degree of acquired resistance was quantified *a posteriori*, by exposing males to a secondary infection. Sigmoid display rates and female preference for males differed for males of different resistance groups after challenge infection only. This difference was due to resistant males displaying more than non-resistant ones. No differences were detected in male orange colour, but this may be because colour needs a longer time than ten days to be recovered and adjusted. The results show that the level of acquired resistance affects sexual display and attractiveness in guppies. They suggest that once an effective immunity is built up by a male, he can afford to incur higher costs for sexual characteristics, whereas a male that lacks the ability to build up effective resistance cannot. These costs probably consist of higher energy expenditure and/or higher circulating levels of testosterone, which may be needed to increase display. Priming and effective establishment of an individual's resistance to parasitic infection could eventually result in a higher availability of resources for sexual functions.

Keywords: acquired resistance; guppy; *Gyrodactylus*; handicap; sexual selection

1. INTRODUCTION

Handicap models suggest that male secondary sexual characters may represent genetic quality signalling traits (Zahavi 1975, 1977; Grafen 1990). Sexual characters are handicaps because they are costly, but more so to individuals of low than of high genetic quality. This cost difference ensures reliable signalling of quality.

As Hamilton & Zuk (1982) suggested, resistance to parasites may be one important genetic quality revealed through exaggerated secondary sexual characters. Therefore, females who base their mate choice on such characters could gain direct benefits (i.e. avoidance of contagion; good parental care) as well as indirect benefits (i.e. resistance genes for their offspring) from their discriminatory preference. Many observational and experimental studies support Hamilton & Zuk's hypothesis by revealing a negative relationship between a male's parasite load and the showiness of his sexual characters (e.g. Møller 1988, 1990; Clayton 1990; Milinski & Bakker 1990; Zuk *et al.* 1990*a,b*; Houde & Torio 1992; Saino & Møller 1996; for reviews, see Clayton (1991) and Møller & Saino (1994)). These same studies also provide evidence for a discriminatory preference of females for the showiest and least infected males.

The immunohandicap hypothesis offers a mechanistic extension to Hamilton & Zuk's handicap version (Folstad & Karter 1992; Wedekind & Folstad 1994). It is based on

the fact that circulating testosterone enhances the expression of many male secondary sexual characters and behaviours, but at the same time suppress the immune system. Thus, males producing well-developed secondary sexual characteristics may be indicating their superior parasite resistance. Ornaments represent handicaps that potentially reveal a male's ability to allocate sufficient resources to both immunocompetence and the maintenance of sexual characters. One prediction made by this hypothesis is that males expressing more exaggerated secondary sexual characters will show lower levels of current immune response, and indeed there are now a few recent studies supporting this prediction (Zuk *et al.* 1995; Møller *et al.* 1996; Skarstein & Folstad 1996; Saino *et al.* 1997).

All studies outlined above provide evidence for an existing relationship between secondary sexual characters and resistance to disease, measured as parasite load and/or immune response level in hosts that are naturally or experimentally exposed to parasites. Resistance to disease falls into two categories: innate (non-adaptive) resistance or immunity, and acquired (adaptive) resistance or immunity, both of which are under genetic control (Roitt *et al.* 1996; Wakelin & Apanius 1997). For hosts that are exposed to parasites and successfully colonized by them, the expression of acquired resistance is the most important way in which the infection load may be controlled. An individual's level of acquired resistance to a certain parasite

is subject to great variation during the course of exposure and infection. This is because every acquired immune response needs to be induced and developed by the initial exposure to parasites, i.e. it needs priming. The primary and other subsequent immune responses differ qualitatively and quantitatively, i.e. primary responses take much longer to build up, and reach lower levels than subsequent ones (Feldmann 1996; Wakelin & Apanius 1997). During the long building-up time of a primary immune response a host is likely to suffer most from the negative effects of both the weakening parasites and the energy required to activate the immune system. Whereas in subsequent exposures to the same parasite the host will become infected only briefly (if at all), owing to the accelerated and more efficient properties of secondary immune responses. It follows that when a host does not possess an initial innate resistance against certain parasites, it will always face high costs for the required priming of an immune response. Thereafter, hosts that had the genetic ability to build up an effective immune response will be protected by the new level of acquired resistance; thus, they will face lower costs in subsequent encounters with these parasites than hosts without such a genetic ability.

Because of these differential costs that individuals will incur during different stages of resistance development, it can be predicted that costly secondary sexual characters of individual hosts will be adjusted following the handicap principle. In this scenario it means that once males have been exposed to parasites, each individual host will adjust the showiness of sexual characters according to his ability to build and maintain an effective resistance. However, there exist no studies relating the expression of secondary sexual characters to an individual's level of acquired immunity. The aim of this study is to investigate the properties of such a relationship in guppies (*Poecilia reticulata*) exposed to the monogenean parasite *Gyrodactylus turnbulli*.

For the handicap principle to work, the resistance signalled through sexual ornaments must be genetic, i.e. heritable. Genetically based variations in immune response are well-recorded in experimentally controlled studies (Wassom *et al.* 1974, 1986; Wakelin 1975; Wakelin & Blackwell 1988; Møller 1990; Wakelin & Rose 1990; Wakelin & Apanius 1997). Most recorded examples are of continuous variation between extremes of resistance and susceptibility to parasites, although some examples are of discontinuous variation, where a host falls into one or another category. For the guppy host there is experimental evidence that variability in acquired resistance to *Gyrodactylus* parasites is of genetic origin, and thus heritable (Madhavi & Anderson 1985), and that acquired resistance plays a major role in counteracting *G. turnbulli* infections, as only small fractions (<10%) of studied host populations possess an initial innate resistance (Scott 1985a,b; S. López, personal observations). Moreover, variation in acquired resistance appears to be discontinuous and guppies fall into three categories of resistance (Scott 1985a,b).

The guppy is a small, tropical, live-bearing fish with internal fertilization. Colour patterns are expressed only in males and, in the population used here (Paria River, Trinidad), consist mainly of orange (carotenoids) and black spots. Colour area appears to be genetically fixed, whereas expression of colour is individually variable

(Houde 1992), and its expression may be regulated by the male hormone testosterone (Hildemann 1954; Dzwilllo 1962; López 1998). Males court females throughout the year by performing 'sigmoid displays', which consist of vibrating with an arch-bended body in front of a female.

Gyrodactylus turnbulli is an external monogenean parasite of guppies. It attaches with hooks to the host's surface, preferentially on the fins. It has no intermediate hosts and reproduces directly on the host's surface. In guppies from the Paria River (the population used here), *G. turnbulli* is the most and probably the only prevalent macroparasite (54% prevalence in a study by Lyles (1990); S. López, unpublished survey 1996: 60% and 92% prevalence at two different sites). *G. turnbulli* have a negative effect on host fitness. In laboratory infections, high numbers of flukes eventually result in death of hosts (Scott & Anderson 1984; Scott 1985a,b; Lyles 1990). The parasites are also known to inhibit courtship behaviour and reduce orange intensity in male guppies, rendering them less attractive to females (Kennedy *et al.* 1987; Houde & Törjő 1992).

In the present study I test (i) whether the intensity of male courtship display and orange colour is related to a male's level of acquired resistance to *G. turnbulli*, as predicted by the handicap principle; and (ii) whether a female's preference for a mate is affected by existing variability in individual male display and colour. Both the change in male secondary sexual characters and the change in female preference for males exposed to a primary challenge infection are compared between individuals of three different resistance levels.

2. METHODS

Guppies used in this experiment were laboratory-raised, first generation descendants of fish collected from the Paria River in Trinidad. They were all of similar age (4–5 months) and size (males, 14.17 ± 0.54 mm; females, 19.60 ± 0.65 mm) and had never been exposed to *Gyrodactylus* previous to the experiment. I maintained all fish at 23–24 °C on a 12 h day, 12 h night cycle and fed them TetraMin food flakes daily.

I obtained *Gyrodactylus turnbulli* from a laboratory culture kept on mixed guppies (from various populations), which was derived from a few infected wild-caught Paria guppies six months previously.

(a) *Experimental design*

Forty-three males and ten virgin females were used as experimental subjects. To test for the effect of acquired resistance on male sexual displays and female preferences, two observation trials were carried out for each male individual: one trial before a challenge infection with *Gyrodactylus*, and another one 10 d after the last day of this challenge. The challenge consisted of exposing males to *Gyrodactylus*, as described below, and disinfecting them by chemical treatment after 7 d. Thus, observation trials were separated by 17 d, and fish were never actually infected at the time of the observation trials. At day 7, the challenge infection varied between 7 and 24 parasites per male. Only one individual appeared not to be infected, probably as a result of an initial genetic resistance of this fish. This individual was therefore excluded from the experiment.

Both observation trials were conducted in the same experimental tanks (40 l, 45 cm × 30 cm × 30 cm). I set up two

experimental tanks in exactly the same way, each containing a small moss-plant and an airing stone in the centre, and two small (18 cm × 12 cm × 12 cm) transparent inset tanks at their distant ends. These small inset tanks were completely submerged in the water content of the experimental tank and were covered with perforated plastic lids, which were held in place by the weight of a stone. The experimental tanks were divided into two half-tank compartments (each compartment containing a small inset tank) by drawing a line on the glass.

Five virgin females were placed in each experimental tank and were given 3 d to habituate before starting an observation trial. I tested males by placing each one in the small inset tank at one end of the experimental tank. The side to place the male in was chosen at random. An unfamiliar female was placed in the inset tank at the other end. Each male was given 15 min to acclimatize before starting observations. Observation intervals lasted 10 min. The following behavioural variables were recorded: the number of 'sigmoid displays' performed by each male, the number of 'glides' of virgin females towards the displaying male and the 'time spent' by the majority of these females (i.e. three or more) in the half-tank compartment containing the male. Since guppies tend to aggregate around conspecifics, the unfamiliar female at the opposite end of the tank was used to control for such an effect. 'Gliding' towards a male and 'time spent' with a male are both measures for female preference. Although the latter is a very general measure of preference, 'gliding' is a more accurate one, as it often results in cooperative copulation. Each male was tested once more in the same way in the second experimental tank containing five other virgin females. For the data analyses, the recorded behavioural variables were averaged over both tests for each male individual and were expressed as counts per 10 min. I further calculated an index for the attractiveness of a male's actual display, which was expressed as the fraction of female gliding responses per male sigmoid display.

All observations were conducted on 11 consecutive days, in the morning between 0800 and 1230, the time when guppies are usually most active.

Following an observation trial, each male was photographed on the afternoon of the same trial day, in order to measure orange colour intensity later. Immediately after taking the photographs, males were exposed to a primary challenge infection (after first trial) or to a secondary infection (after second trial). For the challenge infection, ten individuals were selected at random to be only sham-infected (see below) and to serve as controls.

Following an infection each male was maintained singly in a squared, transparent 1 l plastic bottle containing a moss-plant. They were fed daily on Tetramin food flakes, and water was exchanged every 3–4 d.

(b) *Photographing and measuring colour*

The colour intensity of male orange spots was determined from photographic slides using a densitometer. Guppies were photographed in the following way. Each male was placed in a Plexiglas container (3 cm × 3 cm × 1 cm), filled to a depth of 2 cm with water. An adjusted black, soft sponge was introduced and the guppy was gently squeezed between the container and the sponge. A plastic-coated standard colour card (with an orange, a black and a white standard) was immersed and placed above the guppy. The fish were photographed twice in exactly the same position, once with a fitted orange-pass filter on the camera lens (an HTMC Filter hama, which transmits light only above 545 nm), and once without the filter. All slides were taken with a standard lens fitted with an extension tube and a close-up

lens (+4). Light was provided by a flash placed at a 45° angle, 35 cm from the object. The distance between object and film was set at 36 cm, the aperture used was f/16. Slides were taken on Kodachrome 64 ASA professional film.

Each slide was put through a densitometer (SEESCAN Imaging System with Precision Power Supply) to determine colour saturation of orange spots. The optical density (OD) values obtained from these readings are a measure for the proportion of light being transmitted through the slide when light falls on it. The smaller this transmission is, the greater the absorption of light by the slide and the more saturated the colour of this spot. The transmission through the colour standard and the orange spots was obtained for each slide. White and black standards were used for calibration of the densitometer; OD values obtained for orange standards were subtracted from the values of orange spots to correct for possible differences between films. For all spots on the fish, an average OD value was calculated. With these numbers I calculated an index that was aimed to produce a single value expressing the transmission of the colour of interest (orange): $\text{transmission} = (F - C) / (F + C)$, where $C = \text{OD}$ average of the clear image and $F = \text{OD}$ average of the filtered image. The absorption of orange colour was then calculated: $\text{absorption} = 1 - [(F - C) / (F + C)]$. This value was used as a measure of orange saturation for the data analyses.

(c) *Infection and disinfection*

Males to be infected were placed individually on a small Petri dish (diameter = 3 cm) with just enough water to cover the fish when holding the dish inclined. In this position the movements of the fish slowed down, which allowed me to put them in contact with a small piece of infected fin from a donor, observing the action under the dissection microscope. The donor fin was removed immediately after one parasite passed over to the recipient fish, which typically occurred within a few seconds to 5 min. Control fish were sham-infected by holding them the same way in the Petri dishes except that they were not exposed to parasites. The donor pieces were obtained by killing one heavily infected guppy several hours before the experimental infection.

All fish were treated with the antiparasitic medication 'Life Bearer' (Aquarium products, 0,0-dimethyl 1-hydroxy 2-trichloromethyl phosphate) to remove parasites on day 7 of the challenge infection and on day 22 of the secondary infection.

(d) *Classification in resistance groups*

I monitored the course of the secondary infection by counting *Gyrodactylus* fluke numbers in regular time-intervals of 3–4 d. After 21 d of monitoring, individual guppies could be assigned to three different resistance categories, which were characterized by the number of parasites present on one fish (figure 1). This pattern of acquired resistance is consistent with preliminary observations on the same population and with the results obtained from other populations (Scott 1985*a,b*). Nine males carried no parasites and were called 'resistant males' (r1), nine fish had low numbers of flukes and were called 'partly resistant males' (r2), and 14 fish had very high numbers of parasites and sticky fins and were called 'non-resistant males' (r3). These non-resistant males are obviously suffering under the detrimental effects of their high parasite loads and would die within a few days if left untreated. The three identified resistance groups may represent a spectrum of continuous resistance, with r1 fish and r3 fish being the two extremes of this spectrum.

Figure 1. Number of *Gyrodactylus turnbulli* per fish, 21 d after a secondary experimental infection. Each point marks one fish individual. Fish can be classified in three resistance groups: r1, resistant hosts; r2, partly resistant hosts; and r3, non-resistant hosts.

Because I determined a male's resistance only after the experimental measurements, testing order was completely randomized and I was naive of a male's resistance degree during recordings.

(e) Statistical analyses

Analyses of variance (ANOVAs) were used to test for the effect of acquired resistance (effect of r1, r2 and r3 males) on male secondary sexual trait variables and on female preference variables before and after challenge infection. Changes in sexual trait variables and changes in preference variables were expressed as differences, i.e. variable value after challenge infection minus paired variable value before challenge infection. ANOVAs were then used to test for effects of groups (control, r1, r2 and r3 males) on these difference variables. Two lower-case numbers are attached to the F -values of ANOVAs, which represent the degrees of freedom (d.f.) and the total number of tested males (N), respectively, i.e. $F_{d.f.,N}$. Where significant effects were found, Tukey *post hoc* comparisons were performed to detect differences between group pairs.

Data on 'time spent' were not normally distributed and had to be arcsin transformed for the analyses. All p -values represent one-tailed probabilities, because the tested hypotheses made directional predictions.

3. RESULTS

Male sigmoid display rates were not different between resistant groups before challenge infection ($F_{2,32}=0.473$, $p=0.35$), but they were significantly different after challenge (figure 2a). This difference was due to resistant males (r1) displaying more than non-resistant ones (*post hoc* pairwise comparisons: r1–r2, $p=0.042$; r1–r3, $p=0.06$; r1–pooled r2/r3, $p=0.019$). A trend effect was found for change in sigmoid display rate (displays after challenge

Figure 2. Behavioural variables recorded before (shaded bars) and after (open bars) challenge infection: c are sham infected control males ($n=10$), r1 are resistant males ($n=9$), r2 are partly resistant males ($n=9$) and r3 are non-resistant males ($n=14$). Standard errors are indicated. (a) Male sigmoid display counts per 10 min. Sigmoids after challenge (open bars) are significantly different between r1, r2 and r3 ($F_{2,32}=2.651$, $p=0.04$). (b) Female glides towards a male per 10 min. Change in glides (difference) differs significantly between groups ($F_{3,42}=7.73$, $p<0.001$), and glides after challenge (open bars) are significantly different between r1, r2 and r3 groups ($F_{2,32}=11.32$, $p<0.001$). (c) Male attractiveness expressed as proportion of glides per sigmoid. Change in male attractiveness differs between groups ($F_{3,42}=2.572$, $p=0.035$), and attractiveness after challenge (open bars) is significantly different between r1, r2 and r3 ($F_{2,32}=3.489$, $p=0.02$).

minus displays before challenge: $F_{3,42}=2.0$, $p=0.07$). Male colour spots did not differ in orange intensity between males of different resistances either before or after challenge infection ($F_{2,32}=0.475$, $p=0.35$; $F_{2,32}=1.232$, $p=0.15$, respectively), nor did individual colour-change differ between experimental groups ($F_{3,42}=0.117$, $p=0.47$).

The amount of time spent by females next to a male did not differ between male resistance groups either before or after challenge infection ($F_{2,32}=0.53$, $p=0.33$; $F_{2,32}=1.363$, $p=0.13$, respectively), and change in time spent by females with a male did not differ between experimental groups either ($F_{3,42}=0.104$, $p=0.47$). Female preference measured as glides towards a male was not different between male resistance groups before challenge infection ($F_{2,32}=0.802$, $p=0.25$), but it was significantly different after challenge infection, and so was the change in glides between experimental groups (figure 2b). This difference is marked by a much higher female preference for resistant males (*post hoc* pairwise comparisons: r1–r2, $p<0.001$; r1–r3, $p<0.001$; r1–pooled r2/r3, $p<0.001$). The fraction of female glides per male display differed significantly between resistance groups after challenge infection only (but not before challenge: $F_{2,32}=0.881$, $p=0.23$) and its change was also significantly different between experimental groups (figure 2c). Again this difference was due to resistant males (r1) being more attractive than non-resistant ones after challenge (*post hoc* pairwise comparisons: r1–r3, $p=0.018$; r1–r2, $p=0.16$; r1–pooled r2/r3, $p=0.011$).

4. DISCUSSION

The main result of this study shows that sexual display rates of resistant males are higher than display rates of non-resistant males after a challenge infection with *G. turnbulli*, and that female guppies preferentially choose resistant males over non-resistant ones after the challenge. Male sexual display is probably one important cue upon which females here based their choice, as the fraction of 'female glides per sigmoid display' was significantly higher in front of the resistant males.

This result is consistent with the handicap principle, because it shows that high-quality males, i.e. males with the genetic ability to establish an effective resistance against *G. turnbulli*, invest more in sexual display than non-resistant males. However, this effect was only found after briefly challenging males with *G. turnbulli*, disinfecting and allowing them to recover for ten days. Before having exposed the same males to parasites, no relation between the amount of sexual display and their ability to acquire resistance was detected, nor did females show any discriminatory preference. This finding suggests that in host populations, where the expression of acquired immunity is the most important way to counteract parasites (because an initial innate resistance is rare or absent—in the studied population just one out of 43 males may have possessed an innate resistance), the handicap mechanism will only work if most individuals in the population are exposed to parasites. In the present guppy population this is very likely to occur, as *G. turnbulli* prevalence in the wild is high (Lyles 1990; S. López, own unpublished survey 1996) and the main route of parasite transmissions is probably through sexual contacts, which occur frequently.

Why should acquired resistance be reflected in secondary sexual characters only after exposure to parasites but not before? As handicap models propose, secondary sexual characters and behaviours may be costly to express and maintain. Possible costs are reduction of energy resources and essential metabolites for competing demands, such as immune function (Gustafsson *et al.* 1994; Møller 1995), reduced efficiency of the immune system caused by high androgen levels (Folstad & Karter 1992) and, consequently, elevated infection risk and mortality risk arising from parasitism. Individuals who possess an effective resistance against prevalent parasites will pay relatively less costs by expressing exaggerated sexual characters than individuals who lack such a genetic ability. However, an individual's genetic ability to respond immunologically to parasites is not a fixed phenomenon, but subject to great variation during the course of exposure to parasites and infection. In naive hosts this ability (i.e. acquired resistance) needs to be induced by an initial exposure to parasites (called priming). The first or primary immune response that follows needs longer to build up and is less efficient than subsequent immune responses (Feldmann 1996; Wakelin & Apanius 1997). Consequently, any particular host is likely to pay higher costs during the first exposure to parasites than during subsequent ones, due to the prolonged exposure to parasites and the energy requirement of the activated immune system. In fact, individuals that possess the ability to build up a totally effective immune response may remain completely refractory to infection during subsequent exposures to parasites. These properties of acquired resistance may account for the differential increase in sexual display upon challenge, which was found in this experiment: that is, any particular host with the genetic ability to acquire an effective resistance against *G. turnbulli* should be able to invest more resources into sexual traits after having developed and established its resistance, because this new protection decreases or completely removes the risk of reinfection and the obligatory costs that would arise from it. This argumentation explains the increase in display and the resulting display differences between resistance groups after challenge, but still gives no explanation why there are no apparent differences in sexual display between resistance groups before challenge infection. The fact that in the absence of parasites all fish displayed at similar, but not at maximal, rates may be indicating that possessing an unchallenged immune system is generally costly for all individuals, both in terms of fixed maintenance costs and facultative immune activation costs (which depend on infection risk). This kind of reasoning leads to the prediction that resistant males should actively seek for infection, because after exposure to *G. turnbulli* they could perform more sigmoid displays and thus enhance their reproductive success. This may well be the case in this species, since male guppies are almost permanently sexual active, that is courting or harassing females and attempting to mate with them. Such behaviour is particularly risky in this population, because parasite prevalence is high and sexual contacts may increase the risk of becoming infected (López 1998). Resistant males may therefore actively increase this risk by increasing sexual contacts.

The reported differential increase in male sexual display after recovering from challenge stands in contrast

to previous experimental studies, which report differential decreases in male sexual trait expression as a consequence of an immediate parasitic effect and probably a primary host immune response (e.g. Milinski & Bakker 1990; Houde & Torio 1992; Saino & Møller 1996).

It remains unclear what mechanism causes increased allocation of resources into sexual functions after priming and effective establishment of resistance has occurred. However, it could be postulated that the level of acquired immunity determines the allocation of resources via presence or absence of certain immune metabolites (e.g. memory cells). Another possibility is that increased allocation of resources into secondary sexual traits is mediated through androgens (e.g. testosterone), which promote the expression of such traits, and at the same time may have a suppressive effect on the immune system (Wedekind & Folstad 1994). Recent evidence from experiments with rats show that testosterone levels were only decreased during challenge infections, but increase again to the level of control rats during subsequent infections (Biro-Sauveur *et al.* 1994). However, for guppies it is not known whether testosterone has an enhancing effect on male sexual display. Even if a hormone was acting as mediator for resource allocation, the critical question to ask then would be, what are the physiological or immunological changes of a male that has acquired resistance and how are hormones being regulated in respect of these changes? Collaborative investigations by immunologists and ecologists into this subject promise to yield exciting insights about the relation between resistance development and the evolution of sexual traits.

In the present experiment, male orange colour intensity was not affected either by challenge infection or by a male's level of acquired resistance. The lack of a significant effect on this sexual trait may be due to colour needing a longer time to be recovered and adjusted than the ten days given here. Houde & Torio (1992) tested Paria guppies only one day after disinfection from *G. turnbulli* and found that orange colour intensity was affected negatively by infection. The guppies in their experiment were probably still debilitated from infection, whereas the fish here may have had time to recover their colour, but perhaps not enough time for resistant fish to become more colourful. Information on colour adjustment properties would help to design a more appropriate experiment to detect a possible relationship between this male sexual ornament and the development of resistance.

This study provides evidence for an existing relationship between level of acquired resistance and a male's sexual display, as predicted by handicap models, and shows a discriminatory preference by females for males signalling effective resistance. Because male guppies that have successfully acquired resistance are able to display more than 'naive' males or more than males with lower levels of resistance, the study strongly suggests that the establishment and development of resistance could eventually result in higher availability of resources for sexual functions.

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