

Parasite load predicts mate choice in guppies

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Summary. The influence of parasites on mate selection and on a secondary sexual character was studied in guppies (*Poecilia reticulata*). In fish infected with *Camallanus cotti* or *Gyrodactylus*, females were found to prefer males with relatively few parasites, and this preference was associated with a higher display rate in less parasitised males.

Introduction

“Good gene” models of sexual selection (e.g. Trivers 1972; Halliday 1983) have been criticised because of one major flaw – that the genetic variance of the trait chosen would be eliminated after a few generations (Kirkpatrick 1985). However, Hamilton and Zuk’s (1982) model surmounts this problem. The model proposes that the pervasive risk of parasite infection has led to selection for choice of mates possessing traits indicative of resistance. Because of the cyclic nature of host-parasite interactions, the genetic variance of the male’s resistance can be maintained (Hamilton 1982; Anderson and May 1985).

Here we describe a laboratory study of two predictions of Hamilton and Zuk’s model; that female preference varies as a function of the parasite load, and that this preference may be based on characters of health and vigour, and which have been subject to selection for this reason. We investigated the effects of two parasite species *Camallanus cotti* (Fujita) and a *Gyrodactylus* sp.¹ on the

sexual behaviour and mate choice of guppies *Poecilia reticulata* (Peters).

Guppies are particularly suitable for the investigation of this problem, for the following reasons:

a) They are strongly sexually dimorphic, and the males’ genetically polymorphic colour patterns have been shown to represent a balance between sexual selection and local predation intensity (Endler 1983).

b) Female choice has been demonstrated to be more important than male competition in this sexual selection system (Farr 1980; Houde 1987).

c) Male mating success is affected by a variety of factors, including contrast with the background (Endler 1983), degree of carotenoid colouration (Endler 1983; Kodrick-Brown 1985), tail length (Bischoff et al. 1985) and male sigmoid display rate (Farr 1980).

Hamilton and Zuk’s theory concerns parasites which weaken without necessarily killing their hosts, and which interact with the host in relatively long coadaptational cycles. Although its biology has not been fully investigated, the gut dwelling nematode *Camallanus cotti* may be placed in this category (Stumpp 1975). In *Camallanus* species the fish host is infected by feeding on the intermediate hosts, which are small crustaceans (Campana-Rouget et al. 1976; Moravec 1969a, b; Stomberg and Crites 1974). *C. cotti* appears to feed on the fish host’s blood by partially ingesting a section of the intestinal wall. Small numbers of nematodes have no noticeable effect on individual guppies, but large numbers are often fatal (Stumpp 1975). The second parasite, a *Gyrodactylus* sp., is an ectoparasitic monogenean with a direct life cycle which occasionally produces fatal infections if the parasites reach high intensities. However, most fish infected by this parasite recover from an infection after a few weeks (Malmberg 1970).

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¹ The taxonomy of *Gyrodactylus* spp. is extremely confused. The species we used was originally identified as *elegans*, but we have since been informed that this is unlikely. Unfortunately we have no further specimens upon which further identification could be based

Methods

Female preference experiment

Although female choice is important in guppy mating, males are more active and so it is impossible to ascertain female preferences during normal sexual behaviour. Female preferences were therefore tested using males separated from them by perspex partitions. The 16 female guppies used in this experiment were collected as adults from the Paria river in north Trinidad, a stream with relatively low predation pressure (Endler 1978). Since the wild caught males were insufficiently parasitised and because the wild females would have had prior exposure to them, the 30 males used in the experiment were collected from a large semi-natural population of guppies living in a pool in a heated greenhouse belonging to the Oxford University Botanic Garden. Although it originated from pet shop stocks, the population has largely reverted to the appearance of wild fish. Preliminary dissections of 22 individuals had revealed *C. cotti* at a prevalence of 91% with an intensity of 1–5, but no other parasites.

The choice tests were carried out in a 55 l aquarium which had been divided into three by transparent perspex partitions. A mixture of 50% black and white gravel was attached to the back, sides and floor of the aquarium, to control for background colour patterns (see Endler 1983). Lighting was from three 40-watt fluorescent lamps 1.5 m above the main aquarium, and all the aquaria were maintained at a temperature of 25° C. A small aquarium containing two females from the Botanic Garden pool stood behind the centre of the back wall of the experimental aquarium to maximise the likelihood that the time spent near males could be attributed to sexual attraction rather than merely a tendency to aggregate with conspecifics. Each of 15 pairs of males was tested with all 16 females in turn, in balanced order. Pairs of males were matched for size, tail length and colouring to minimise female choice on the basis of criteria other than parasite load.

At the beginning of a set of observations the two females were placed in the small aquarium, and one of each of a matched pair of males was placed in each of the end compartments of the experimental aquarium. The first experimental female was then placed in the central compartment of the experimental aquarium, and after 5 min black perspex partitions which prevented vision between the compartments were removed. Observations began after a further 10 min acclimation. For 20 min the time and position of the experimental female were recorded each time she changed positions as follows: L(left), C(centre), R(right), and LT and RT if the female was within 2 cms of the left and right males respectively. The number of sigmoid displays (see Baerends et al. 1955) performed by each male when the female was on its side was also counted. After half of the females had been observed with one male pair the males' positions were exchanged, and the observations completed with the rest of the females. Each male pair was then dissected to establish parasite loads, freeze dried and weighed. The nematodes were counted and volumes estimated from their lengths and diameters. At the end of the experiment all the females were dissected, but apart from two instances of mild protozoan (*Chilodonella* sp.) infections they were free of parasites.

Preference scores were obtained by assessing the females' relative allocation of time to various positions in the aquarium. LT, L, C, R and RT were given arbitrary values of –2, –1, 0, 1 and 2 respectively, and weighted mean positions for a given female with a given male pair were calculated. The weights were either a) the mean time spent in each position (bout length), or b) the total time spent in each position as

a fraction of the observation time. "Relative sigmoid rate" was the ratio $(L - M)/(L + M)$, where L is the display rate of the less parasitised male and M is the display rate of the more parasitised male.

Male sexual behaviour experiment

In the second experiment, the fish were allowed to interact directly so that the effects of parasites on normal sexual behaviour could be observed. Unparasitised guppies from a pet shop were experimentally infected with *Gyrodactylus* by placing them in an aquarium with two heavily parasitised goldfish (*Carassius auratus* L.). Control guppies were kept at the same densities with unparasitised goldfish. After 2 days a low level of *Gyrodactylus* infection was observed on the experimental fish, and the goldfish were removed from both aquaria. Following the initial infestation, and then at weekly intervals, four aquaria were set up, each containing four unparasitised females (unfamiliar to all the males) and two each of the control and experimentally infected males. Each male was observed and the number of sigmoid displays recorded for 15 min, with order of observations determined by balanced design. After each observation session the males were anaesthetised with MS-222 Sandoz, and scored for numbers of *Gyrodactylus*.

Results

Female preference experiment

The females' preferences for males were related to the number of parasites each carried (Table 1). In all but one of the male pairs, the females stayed for longer bouts with the male with fewer nematodes. This is significant by the conservative two-tailed Binomial test ($P < 0.001$). Females spent longer altogether with males with fewer parasites in 10 out of the 15 male pairs ($P = 0.302$). Individual females consistently preferred the less parasitised male (bout length 14/16, $P = 0.004$; total time 12/16, $P = 0.706$, two-tailed Binomial tests). However, although the nematodes varied a great deal in size (from 1.6 mm to 6.2 mm long, comprising stage 4 larvae and adults of both sexes), there was no relationship between parasite load measured by volume, and preferences measured by either bout lengths ($P = 0.302$) or total times ($P \sim 1$). The same pattern arises when the rate of sigmoid displays is compared within pairs of males. With the parasite load measured in numbers, the less parasitised male performed a relatively higher sigmoid display rate in 12 out of the 13 cases where sigmoid numbers differed ($P < 0.001$, two-tailed Binomial test) but this relationship applied in only 7 out of 14 male pairs if the parasite load is measured by volume.

Females were heterogeneous with respect to preferences calculated on bout time ($F[15,210] = 1.7$, $P < 0.05$) and total time ($F[15,208] = 2.68$, $P < 0.001$) (Table 2).

Table 1. Pooled female preferences for each pair of males in relation to numbers of *Camallanus cotti* parasites. The data in this table are arranged so that a positive score indicates a preference for the less parasitised male (for calculation of preference scores and relative sigmoid rate see text)

Male pair no.	No. of nematodes ^a		Preference scores				Relative sigmoid rate		No. of females tested
			Bout time		Total time		Mean	S.E.	
			Mean	S.E.	Mean	S.E.			
1	0	1	3.31	14.67	8.02	14.78	—	— ^b	16
2	1	2	14.10	7.05	25.83	9.22	18.75	10.08	16
3	1	6	13.63	6.21	7.09	5.78	-13.33	16.52	15
4	1	2	14.44	6.76	22.23	9.02	25.00	17.08	16
5	2	5	11.64	9.02	6.26	10.76	39.58	12.25	16
6	3	4	7.24	9.75	15.64	11.14	33.72	17.36	16
7	1	3	13.12	6.73	-5.85	7.50	0.00	9.13	16
8	1	2	20.59	7.64	30.14	9.57	16.67	12.60	8
9	2	3	2.19	4.94	19.86	7.42	72.05	14.23	16
10	2	3	2.62	7.17	7.03	8.26	2.62	18.54	16
11*	2	2	-14.66	8.22	-9.73	12.56	12.37	21.44	16
12	0	2	5.18	7.22	2.34	8.20	41.07	15.25	16
13*	1	1	4.38	7.72	-3.65	10.31	1.13	18.47	16
14	1	2	3.20	11.93	-5.52	15.46	7.62	18.17	15
15	2	9	6.27	8.82	-1.99	11.90	45.11	14.10	16

Asterisks (*) indicate two pairs in which both males had equal parasite numbers. In these two cases, the preference sign only applies when nematode size is used to break the tie; a positive value indicates preference for the male with an equal number of smaller nematodes

^a Less parasitized male given first

^b Data on sigmoids not taken for the first pair

Table 2. Preferences of individual females pooled across all pairs of males (for calculation of preference scores and relative sigmoid rate see text)

Female	Preference scores				Relative sigmoid rate		No. of male pairs
	Bout time		Total time		Mean	S.E.	
	Mean	S.E.	Mean	S.E.			
1	13.49	9.82	11.82	11.83	41.67	17.01	14
2	-1.30	4.77	-4.82	3.70	13.16	15.33	14
3	-11.76	7.06	-25.06	11.26	9.88	18.07	14
4	10.39	8.57	5.73	11.13	30.42	16.96	14
5	4.14	7.16	-4.68	10.06	20.07	14.22	14
6	2.40	12.07	1.01	11.91	-7.87	16.47	14
7	10.45	7.92	8.02	8.75	28.79	16.03	14
8	0.61	10.76	1.95	14.95	15.10	15.81	14
9	2.01	11.38	13.47	17.31	3.57	23.71	14
10	25.20	8.06	22.63	10.76	38.46	18.04	13
11	18.30	9.99	27.20	12.22	24.40	12.49	14
12	6.21	6.93	18.22	8.12	27.41	17.53	15
13	11.15	9.18	7.50	8.94	26.66	12.99	14
14	15.11	7.45	27.40	9.54	31.75	15.09	15
15	1.01	5.27	6.57	6.10	15.15	19.19	15
16	1.58	9.86	-3.35	8.39	14.22	18.83	15

Male sexual behaviour experiment

The males which had been experimentally parasitised performed significantly fewer displays than the unparasitised controls, ($F[1,47]=57.8$, $P<0.001$)

and there was a clear and highly significant negative correlation ($r=0.939$, $P<0.001$) between numbers of *Gyrodactylus* and male courtship behaviour (see Fig. 1). Males with no parasites courted more than males with parasites, and among those males with parasites, males with fewer parasites courted more than those with many parasites.

Discussion

That female preferences were associated with parasite numbers rather than parasite biomass is to be expected as the pathology caused by *Camallanus* is primarily a function of parasite number rather than of size (Stomberg and Crites 1974), and *Gyrodactylus* adults are all of a similar size.

Our first experiment demonstrated that female guppies preferred males which had fewer parasites and which displayed at a greater rate than males with more parasites. However, since the males were parasitised before the experiment began, one cannot infer a causal mechanism linking display rate with parasite load. The second experiment provides that link by showing that experimentally parasitised males displayed less than controls. It follows that females may be using display rate as an indicator of the males' parasite loads.

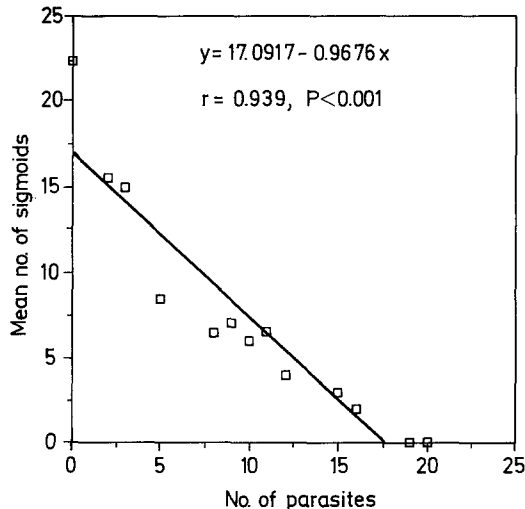


Fig. 1. Graph of mean number of sigmoid displays performed by males of differing parasite loads, fitted with regression line

The significant heterogeneity of female preference observed in the experiment may reflect the genetic variation in preference already shown in guppies (Houde 1987). However, the variance among females can also be attributed to differences in the ovarian cycle, hence female receptivity.

Although these experiments demonstrate that mating behaviour varies with parasite load such that males with no or fewer parasites display at a higher rate than more parasitised fish, this alone is not surprising since one would expect the parasites to have a cost to the host. Since differences in display rates affect female choice even in unparasitised males (Bischoff et al. 1985), the correlation with parasite load could be a secondary effect. What is surprising is that in a semi-natural population of males, parasite load was an *overwhelming predictor* of female preference within a pair of males, irrespective of the extent of their parasite loads. Therefore, these results lend support to Hamilton and Zuk's hypothesis that females prefer to mate with less parasitised males and discriminate on the basis of otherwise disadvantageous secondary sexual characters signalling health and vigour – in this case the males' displays which are known to increase their risk of predation (Endler 1983) (Hamilton and Zuk used plumage colour and song performance in their analysis, but make it clear that other secondary selected sexual traits could be equally valid indicators).

We appreciate that our experiments provide support, but by no means conclusive evidence for Hamilton and Zuk's model of mate choice. We have not tested whether genetic cycles between host and parasite exist, nor if the resistance to the spe-

cific parasites used in this experiment is heritable. The latter is probably since heritability has already been shown to one *Gyrodactylus* species which parasitises guppies (Madhavi and Anderson 1985), and has been shown with nematodes in other animals (Wakelin 1985). As they stand, our results are compatible with Zahavi's handicap model (1975) and also with models incorporating selectively neutral traits as the basis of choice (Lande 1981; Kirkpatrick 1982) if one assumes the negative correlation of display rate with parasite load to be a coincidence. It remains to be seen how generally parasite loads are related to choice in other systems.

Further support for Hamilton and Zuk's model in this system would be gained by demonstrating the females' preference for less parasitised males to be active choice; our results might otherwise be explained by the less parasitised male being more aggressive or more conspicuous than the other. But aggressive behaviour is rare in guppies – and was not observed in the experiments reported here – and it seems unlikely that conspicuousness alone could account for the complexity and energetic expense of the males' display. Further evidence for active female choice would be provided if, for example, the extent of the preference was shown to vary as a function of their stage of vitellogenesis.

What is the selective advantage for a female which mates with less parasitised males? Her own health is unlikely to be affected when an intermediate host is required before the parasite can be transmitted between fish. But where the males' resistance to the parasites is heritable, the females' offspring will be more resistant to the parasite. This may mean a longer lifespan or less vulnerability to predators or pathogens, or avoidance of any direct effect the parasites may have on the fecundity of the offspring. Once established, the process will also mean that male offspring will be chosen by females in preference to less resistant males. These results reinforce the hypothesis that the origin of sexual selection lies in choice of fitness indicators. Resistance to parasites is likely to be the character underlying choice because of the ubiquitous presence of parasites and because the cycles produced by the host-parasite interactions provide the long term variability necessary for the process to be maintained.

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