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Animal behaviour

Imprinting can cause a maladaptive preference for infectious conspecifics

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Recognizing and associating with specific individuals, such as conspecifics or kin, brings many benefits. One mechanism underlying such recognition is imprinting: the long-term memory of cues encountered during development. Typically, juveniles imprint on cues of nearby individuals and may later associate with phenotypes matching their 'recognition template'. However, phenotype matching could lead to maladaptive social decisions if, for instance, individuals imprint on the cues of conspecifics infected with directly transmitted diseases. To investigate the role of imprinting in the sensory ecology of disease transmission, we exposed juvenile guppies, *Poecilia reticulata*, to the cues of healthy conspecifics, or to those experiencing disease caused by the directly transmitted parasite *Gyrodactylus turnbulli*. In a dichotomous choice test, adult 'disease-imprinted' guppies preferred to associate with the chemical cues of *G. turnbulli*-infected conspecifics, whereas 'healthy-imprinted' guppies preferred to associate with cues of uninfected conspecifics. These responses were only observed when stimulus fish were in late infection, suggesting imprinted fish responded to cues of disease, but not of infection alone. We discuss how maladaptive imprinting may promote disease transmission in natural populations of a social host.

1. Introduction

Recognizing and associating with specific individuals confers many benefits: for example, associating with conspecifics can increase mating opportunities and the efficiency of antipredator defences, and associating with kin reduces aggression [1]. Imprinting, defined as the long-term memory of cues encountered during development [2], is a key mechanism involved in this recognition. Typically, juveniles imprint on the sensory cues of nearby individuals and, even during adulthood, may preferentially associate with phenotypes matching this 'recognition template' [3].

Such phenotype matching commonly leads to adaptive behaviours, but it is not infallible. Wild buzzards, *Buteo buteo*, imprint on the colour morph of their mothers and select mates of the same morph, even among the less fit homozygous morphs, reducing the fitness of their offspring [4]. Fishes including guppies, *Poecilia reticulata* [5], and threespine sticklebacks, *Gasterosteus* spp. [6], can imprint on the cues of heterospecifics when raised with them, and as adults show maladaptive social preferences for these heterospecifics.

Regardless of how well their phenotypes match a recognition template, animals should generally avoid associating with individuals infected with directly transmitted diseases. Avoidance of infected conspecifics is based on chemical cues in a number of taxa (amphibians: [7], mammals: [8] and crustaceans: [9]). While such behavioural avoidance can have important implications for individual- and population-level infections [7], the underlying mechanisms

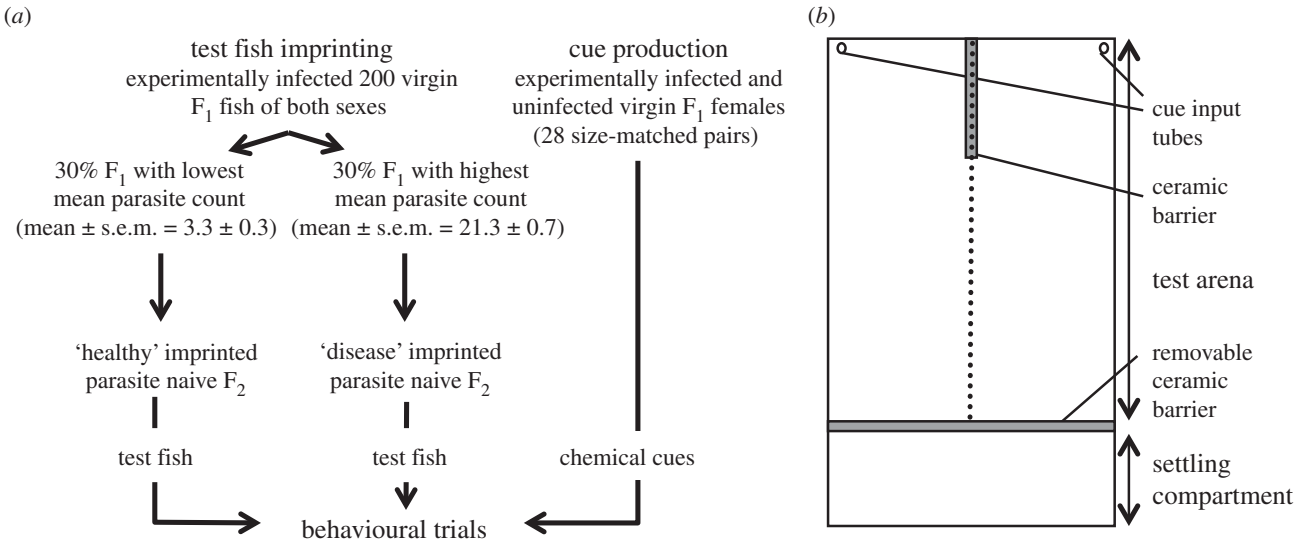

Figure 1. (*a*) We used laboratory-bred descendants of a natural Trinidadian population during test fish imprinting and cue production. (*b*) The tank used to test guppy response to chemical cues of infection. The dotted line was not present, but during video analysis we recorded the proportion of time fish spent on each side of this line.

remain unclear. Infection avoidance behaviour could be innate, or could simply reflect the avoidance of phenotypes that differ from an individual's recognition template.

We used the guppy–*Gyrodactylus turnbulli* host–parasite system to test the hypothesis that the avoidance of infected conspecifics may depend on phenotype matching, and is therefore potentially vulnerable to maladaptive imprinting. We exposed juvenile guppies either to adults experiencing *G. turnbulli*-induced disease, or to *G. turnbulli*-exposed but healthy adults. We then compared the response of these fish as adults to the chemical cues of *G. turnbulli*-infected and -uninfected conspecifics. Importantly, we exposed imprinted fish to chemical cues from fish in both early and late stages of infection to test whether guppies responded to the presence of the parasite in the absence of disease.

2. Material and methods

(a) Fish origin and maintenance

We used first (F_1) and second (F_2) generation laboratory-bred descendants of wild-caught guppies from the Caura River, Trinidad (UTM: 20 P 679527.7 m E, 1180376.4 m N; figure 1*a*). All fish were maintained in dechlorinated 24 ± 1°C water and fed daily. Aquaria housing these wild-caught fish were checked weekly for F_1 fry, which were transferred to rearing tanks. F_1 males and females were separated at six to eight weeks old to ensure virginity.

(b) Test fish imprinting

F_1 fish ($n = 200$) were infected on 'Day 0' with two *G. turnbulli* from the Gt3 laboratory strain, isolated in 1997 from, and maintained since on ornamental guppies (culture fish). Infected culture fish were killed using an overdose of tricaine methanesulfonate (MS222; PHARMAQ UK, Ltd), and F_1 fish were anaesthetized with 0.02% MS222. The tails of the culture and F_1 fish were held adjacently in a Petri dish of dechlorinated water until two parasites had transmitted, as observed using a dissecting microscope. We individually housed these infected F_1 fish in 1 l tanks under standard conditions and counted the parasites infecting each, under anaesthesia and using a dissecting microscope, every other day until Day 9. F_1 fish were then treated with levamisole and confirmed clear of infection during three sequential inspections separated by 4 days. Any fish still infected were re-treated and screened three times.

Once cleared of infection, the 30% (30 males and 30 females) of F_1 fish with the highest mean parasite count over the course of the infection were placed in a breeding tank. F_2 fish born in this tank therefore imprinted on chemical cues of adults that experienced *G. turnbulli*-induced disease. The 30% (30 males and 30 females) with the lowest mean parasite count (difference between the group means ± s.e.m. = 18.0 ± 0.8; $t_{117} = 23.16$, $p < 0.0001$) were placed in a separate breeding tank, where their F_2 offspring imprinted on chemical cues of *G. turnbulli*-exposed but healthy adults (figure 1*a* and electronic supplementary material, S1). F_2 offspring reared in these 'disease' and 'healthy' imprinting tanks for *ca* 7 days (following [5]) before being removed, confirmed uninfected and transferred to one of several rearing tanks.

(c) Cue production

Parasite naive F_1 virgin females from the same population as those used during the imprinting treatment were size matched (±1 mm) to create 28 'stimulus pairs' (figure 1*a*). One fish in each pair was infected and monitored as above. Uninfected fish were handled similarly. For each stimulus pair, we produced chemical cues during both 'early' and 'late' infection (infected for up to 12 days: 'early', or 14 days or more: 'late'; see electronic supplementary material, S2–S4). Chemical cues were obtained by holding fish individually in 500 ml water in food-grade plastic containers for 24 h. Fish were not fed during cue production. Their holding water was subsequently divided into 250 ml aliquots and frozen at −20°C until use.

(d) Behavioural trial protocol

Parasite-naive, sexually mature virgin F_2 fish (27 males and 27 females) were tested for their response to the chemical cues of *G. turnbulli*-infected and -uninfected conspecifics in a modified tank (30 × 60 cm filled to 5 cm depth with 24 ± 1°C dechlorinated water; figure 1). These F_2 test fish were never in the same tank as the F_1 stimulus fish: all cues were thus from unfamiliar individuals. Test fish were placed in the settling compartment to acclimatize for 10 min. After 8 min, chemical cues of infected and uninfected guppies were simultaneously released into the tank via Nalgene® tubing at 10 ml min^{-1} (using flow

Table 1. The final model explaining variation in the proportion of time fish spent associating with the chemical cues of infected conspecifics.

parameter	estimate	standard error	*z*-value	*p*-value
intercept	0.6932	0.5477	1.266	0.21
imprinting (disease)	−0.83	0.7536	−1.097	0.27
stage of infection (late)	−1.8972	0.8564	−2.215	0.027
stage of infection × imprinting	4.3333	1.4496	2.989	0.0028

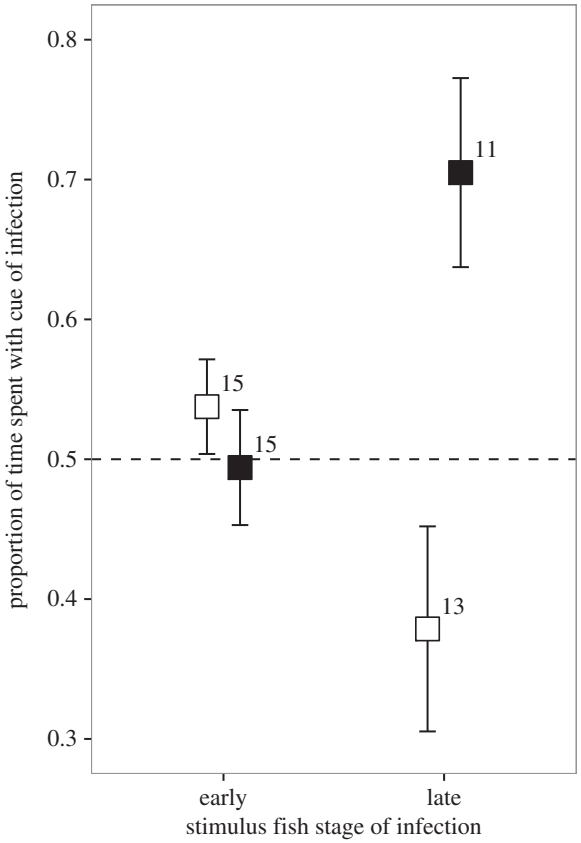

Figure 2. Guppies imprinted on the cues of *Gyrodactylus turnbulli*-exposed but healthy adults (white squares) avoided cues of conspecifics in late infection, whereas those imprinted on the cues of *G. turnbulli*-induced diseased adults (black squares) preferred the cues of conspecifics in late infection. Error bars are the standard errors, and numbers are the sample sizes.

meters: MMA-35, Dwyer® UK). After acclimatization, the barrier was lifted and a 10 min, video-recorded test period began when the fish crossed into the test arena. The proportion of time each test fish spent on the side receiving the infected conspecific chemical cue was quantified using JWatcher™ 1.0 (www.jwatcher.ucla.edu) by an observer blind to the treatment. The test tank was washed with 70% alcohol and rinsed between trials. Test fish sex, imprinting treatment (diseased or healthy), chemical cue used (early or late infection) and the tank side to which each cue was introduced were changed between trials using a Latin square design.

(e) Data analysis

We used a generalized linear mixed model with binomial error family and logit link function in the *lme4* package in R (3.0.2; [10,11]) to test for differences in the proportion of time disease- and healthy-imprinted F_2 test fish spent associated with the chemical cues of infected conspecifics. The model included fixed effects: stimulus fish parasite count, stimulus fish infection stage (early or late), test fish imprinting (disease or healthy), test fish sex and length, tank side that received the cue of infection, and two-way interactions between sex, stage of infection and imprinting. A random term controlled for the fact that each stimulus pair contributed cues to more than one trial (see electronic supplementary material, S3). Model refinement involved stepwise removal of non-significant terms to minimize Akaike's Information Criterion.

3. Results

Test fish showed no preference between the chemical cues from uninfected and infected stimulus fish in early infection (table 1 and figure 2). Test fish avoided cues from stimulus fish in late infection (stage of infection main effect; table 1), but this effect depended on imprinting: healthy-imprinted test fish spent more time associated with the cues of uninfected conspecifics, whereas disease-imprinted test fish spent more time associated with the cues of *G. turnbulli*-infected conspecifics (stage of infection × imprinting interaction: table 1 and figure 2). We found no effect of fish size or sex. The random term explained 12.2% of the variance in the data.

4. Discussion

Our results support the prediction that healthy-imprinted fish avoid the novel chemical cues of diseased fish, analogous to the neophobia guppies have evolved in response to predation pressure [12], whereas disease-imprinted guppies are attracted to cues that match their recognition template [5]. The difference in test fish responses to cues of early and late infection is likely due to changes in cue concentration or composition indicative of disease. The identity of these chemical cues remains unclear, but because F_1 fish used for imprinting were parasite-free for at least one month before the F_2 test fish were born, the cues cannot have come from the parasites. Several gene expression differences have been observed between salmonids varying in their response to gyrodactylid infection, as well as phenotypic differences in humoral immune system response and the secretion rate and composition of mucus (reviewed by Bakke *et al*. [13]); any such change could contribute to differentiated 'healthy' and 'disease' cues.

The observed behavioural effect of disease imprinting is likely maladaptive: healthy, parasite-naive individuals preferentially associated with the cues of conspecifics infected with a directly transmitted disease. It is possible that the degree to which imprinting affects avoidance behaviour depends on the local prevalence of gyrodactylid parasites, and their effect on their hosts, both of which are highly

variable between guppy populations [14]. The maladaptive imprinting we observed may be more likely in low prevalence populations where the overall risk of infection is lower. Indeed, our test population was gyrodactylid-free during both collection and a previous survey [14].

Clearly, the ability to remember and respond to the cues of infected conspecifics is not, in itself, maladaptive. In fact, it may have led to the evolution of innate avoidance behaviour in high prevalence populations: in other taxa, imprinting can lead to lifelong gene expression changes [2], and such gene expression-dependent behaviours can be heritable [15]. Corroboratively, guppy populations do display evolutionary divergence in the degree to which they use phenotype matching, self-referent phenotype matching and prior experience in kin recognition [16].

In summary, our results suggest that avoidance of disease cues is not purely innate, but can be influenced and even overridden by maladaptive imprinting. If so, imprinting-mediated social behaviour may play an important role in the infectious disease transmission dynamics of natural populations.

Ethics. This work was conducted under UK Home Office licence (PPL 30/2876) and approved by the Cardiff University Animal Ethics Committee.

Data accessibility. Raw data are available from Dryad http://dx.doi.org/10.5061/dryad.b2110.

Authors' contributions. J.F.S. designed the study, analysed the data and wrote the manuscript. Both authors collected data, made revisions, gave final approval for publication and agree to be accountable for all aspects of the work.

Competing interests. We have no competing interests.

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