

Assortative interactions and the evolution of cooperation during predator inspection in guppies (*Poecilia reticulata*)

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ABSTRACT

One possible mechanism for the evolution of cooperation/altruism is assortative interactions, in which cooperators interact with each other by choice and cheaters are forced to interact with each other by default. This mechanism has been regarded as implausible in the past, but more recent models have revealed its likelihood when cooperative behaviour is a quantitative trait that can be observed directly by members of the population. Assortative interactions are likely to occur in guppies (*Poecilia reticulata*) because they cooperate in the context of predator inspection, their inspection behaviour is a quantitative trait, and they are known to monitor each other's behaviour and choose to associate with fish that inspect. Despite these pre-conditions, our experiments failed to demonstrate assortative interactions in free-swimming guppies. We offer our negative evidence as an incentive for others to look for what remains a theoretically plausible mechanism for the evolution of cooperation/altruism.

Keywords: altruism, cooperation, predator inspection.

INTRODUCTION

Cooperative and altruistic behaviours are of fundamental importance not only to behavioural ecologists, but also to psychologists, anthropologists, economists and political scientists (Axelrod, 1984; Dugatkin, 1997, 1999; Sober and Wilson, 1998). One possible mechanism for the evolution of cooperation/altruism is assortative interactions, in which cooperators recognize and interact with each other by choice, whereas cheaters are forced to interact with each other by default (Wilson and Dugatkin, 1996). This mechanism has been considered implausible for two reasons: the difficulty of distinguishing cooperators from cheaters (labelled the 'green beard' effect by Dawkins, 1976), and the difficulty of finding cooperators when they are at a low frequency in the population (labelled the 'problem of origination' by Wilson and Dugatkin, 1996).

If cooperative behaviour can be observed directly, as is the case for many types of cooperation (Dugatkin, 1997), the 'green beard' dilemma disappears. In addition, the 'problem

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of origination' can be overcome if cooperative behaviour is a quantitative trait rather than a discrete trait that originates at mutation frequency (Wilson and Dugatkin, 1996; Roberts and Sherratt, 1998). A rare cooperator may have difficulty finding another rare cooperator, but an individual who is above average for cooperation can easily find another above-average individual, even if the average degree of cooperation in the population is very low.

Wilson and Dugatkin (1996) constructed a model that examines cooperation when traits are continuous (rather than discrete categories, such as 'cooperator' and 'cheater') and players are not forced to choose partners randomly. Cooperation and defection were made continuous traits by examining behaviours in which the benefits are shared by all group members, while the costs are paid by cooperators alone. If the costs (C) and benefits (B) are continuous, degrees of cooperativity can be modelled by considering how great a cost individuals are willing to pay, which creates a distribution of $B - C$ values. Non-random partner choice was incorporated by considering a range of cognitive abilities and the effects of such variables as group size.

The results of Wilson and Dugatkin (1996) demonstrate that both non-random partner choice and a continuous distribution of traits increased between-group variance and hence favoured the evolution of cooperation. The extent of cooperation uncovered depended on the particular assumptions made about cognition, but incorporating even the most basic elements of memory and individual recognition favoured cooperation. Using the appropriate animal system, many of the predictions of this model could be tested.

One system particularly well-suited to study assortative interactions and the evolution of cooperation is predator inspection behaviour (Pitcher *et al.*, 1986; Pitcher, 1992). Predator inspection has been documented in many species of fish (Pitcher, 1992; Dugatkin, 1997) and occurs when some individuals break away from a group of fish and slowly approach a potential predator to obtain various types of information (Pitcher *et al.*, 1986; Dugatkin and Godin, 1992; Pitcher, 1992). Although the precise type of cooperation (i.e. reciprocal altruism or by-product mutualism) that inspectors display is still a matter of debate (Connor, 1996; Dugatkin, 1996; Milinski, 1996; see Dugatkin, 1997, for a review), several aspects of predator inspection in guppies (*Poecilia reticulata*) satisfy the prerequisites for assortative interactions identified by theoretical models. In particular, predator inspection is a quantitative trait with reliable differences among individuals (Dugatkin and Alfieri, 1991a), and guppies are known to monitor each other's inspection behaviour and to prefer inspectors as associates in dichotomous choice experiments (Dugatkin and Alfieri, 1991b).

Dugatkin and Alfieri (1991b) designed a series of experiments to examine whether guppies remember the behaviour of their associates during predator inspection and subsequently prefer to associate with the more cooperative individual. In this work, three guppies were placed in the experimental apparatus 'lanes' for the fish to swim in (i.e. no physical contact among fish). Adjacent to the experimental tank was a tank containing a predator, and predator inspection was taped for 2.5 min.

After an inspection trial, guppies were placed into 'preference tanks'. The fish from the centre lane of the inspection trial (the 'centre' fish) was placed into a tank placed between two smaller side chambers. The two fish from the side lanes (the 'side' fish) were placed into the smaller side chambers. The position of the centre fish was noted every 5 s. The results were strikingly clear: the centre fish preferred the side fish which had the average position closest to the predator during the inspection trial – that is, the more cooperative of the side fish. Furthermore, regardless of whether the centre fish itself was a cooperator or a cheater, it preferred to associate with cooperative co-inspectors. Similar results were obtained when

the 'inspection' and 'preference' parts of a trial were separated by 4 h, suggesting that fish can remember this information for significant periods of time. In a similar vein, Milinski *et al.* (1990) found that inspecting sticklebacks built up 'trust' with one another over time, again supporting the notion that partner choice may not be random. Given what appears to be a good fit between the assumptions of our model and what we already know about inspection in guppies, here we tested whether assortative interactions occur among larger, free-swimming groups of male guppies periodically exposed to the sight of predators over the course of many days.

MATERIALS AND METHODS

Twenty-one groups (10 experimental and 11 controls) of six guppies each were tested. Each group consisted of six male guppies descended from a population of fish originally caught in the Paria River, Trinidad, West Indies, in the winter of 1996. Each male in a given group was drawn from a different stock tank to minimize genetic relatedness between individuals within groups. All males in a group were within 10% of each other's body length to minimize effects of size on predator inspection and assortative interactions.

Each run of the experiment was composed of two parts. At the start of part one, 18 male guppies were kept in individual, clear cups (that held 177.4 ml of liquid) filled with water to allow an observer to sketch male colour patterns, thus permitting individual recognition of all fish. After sketching colour patterns, the 18 fish were kept in the individual cups, which were placed on metal rods and hung in a single 37.9 litre tank to allow visual contact for 3 days. On days 4–6, the predator inspection tendency of each male was measured once a day by placing it by itself in a 37.9 litre tank with a mirror placed along one side (Fig. 1A). The mirror created an image of another fish that inspected in the exact manner of the fish being tested. At the far end of the tank was a smaller aquarium, concealed by an opaque partition, containing a prawn (*Macrobrachium* spp.), which is the main aquatic predator of guppies in the Paria River (Houde, 1987). The guppies were allowed to acclimatize to the tank for 5 min, after which time the partition was removed. The behaviour of each guppy was recorded for 2 min using a video camera mounted 55 cm over the test tank. An average inspection score for each fish was calculated for each day. To obtain inspection scores, the 37.9 litre tank was divided into 10 sections of equal length (section 10 being closest to the predator and section 1 being furthest from the predator) and the position of the guppy was noted at 15 s intervals during each trial. Each guppy was tested three times to determine the consistency of individual behaviour and the reliability of individual differences. As in previous experiments (Dugatkin and Alfieri, 1991a), guppies were consistent in their inspection scores across trials (repeated-measures analysis of variance: $F_{2,377} = 0.905$, $P > 0.4$).

Part two of the experiment began on day 7 and used only the six fish with the highest and the six fish with the lowest inspection scores from part one. Two 75.8 litre tanks were stocked with three fish each drawn at random from the 'high' group and three fish drawn at random from the 'low' group, to ensure variation in predator inspection behaviour within each tank and to minimize differences between tanks. Fish drawn from the 'high' group inspected the predator significantly more closely than fish drawn from the 'low' group (unpaired t -test: $t_{124} = 12.353$, $P < 0.0001$). The mean inspection score for high inspectors was 8.0 (i.e. mean position of section 8.0), and the mean inspection score for low inspectors was 5.2 (i.e. mean position of section 5.2). A 9.5 litre tank with the prawn

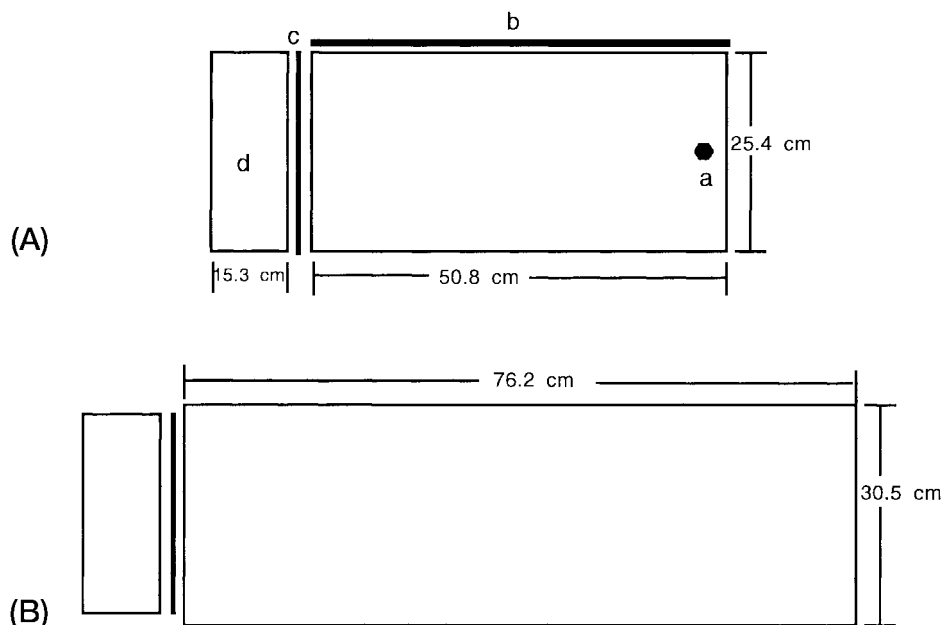


Fig. 1. (A) An overhead view of the 37.9 litre tank used in mirror trials: (a) artificial plant, (b) mirror, (c) opaque partition, (d) 9.5 litre tank containing prawn predator. A video camera was mounted above the tank and inspection behaviour was taped for 2 min after the opaque partition was removed. When data were extracted from the videotapes, the tank containing the guppy was divided into 10 equal sections, with section 10 being closest to the prawn predator and section 1 being farthest away. (B) A top view of the 75.8 litre tank used for nearest-neighbour measurements on days 4–7 (part two of the trials).

predator was placed next to one of the 75.8 litre tanks and an empty 9.5 litre tank was placed next to the other, which served as a no-predator control (see Fig. 1B). On the morning of days 7 and 9, the opaque partitions separating the 75.8 litre tanks and 9.5 litre tanks were raised for 20 min, exposing the guppies in the experimental tank to the sight of the predator. Predator inspection presumably occurred in this tank but was not actually measured during this part of the experiment. The degree of assortative interactions was measured in each 75.8 litre tank by an observer behind a blind, who used focal animal sampling to record the nearest neighbour of all six guppies (distinct male colour patterns made individual recognition straightforward). Nearest neighbours were recorded three times a day on days 7–10.

Parts one and two were performed 11 times, although one experimental trial had to be removed from the analysis (due to an error in protocol), resulting in 10 experimental and 11 control trials. A one-way analysis of variance found no significant differences in mean inspection scores between the 21 groups tested (mean square = 3.801, $F = 1.423$, d.f. = 20, $P > 0.1$), signifying that our experimental design was successful in minimizing variation among groups. Running predator trials with no-predator controls in parallel minimized other confounding variables. If assortative interactions occur in guppies in response to predator inspection, we would expect a positive correlation between the inspection score of

each individual and the inspection score of its nearest neighbour in the experimental tanks, but not in the no-predator controls. We also might expect a stronger correlation in the experimental tanks on the days they were exposed to predators (7 and 9) than on the days they were not (8 and 10).

RESULTS

No evidence was found for assortative interactions. Purely random assortment would result in a line of best fit with a slope of -0.2 when the inspection score rank of an individual was plotted against the average rank of its nearest neighbour. The expectation of a slope of -0.2 (as opposed to 0) is based on the fact that, if it chose at random, first ranked fish would choose between fish ranked 2, 3, 4, 5 and 6 (mean rank value of = 4), whereas sixth ranked fish would choose between fish ranked 1, 2, 3, 4 and 5 (mean rank value of = 3). Similar calculations for second, third, fourth and fifth ranked fish produce a slope of -0.2 when the inspection score rank of an individual is plotted against the average rank of its nearest neighbour. Only two of the ten experimental tanks had slopes that were significantly different from -0.2 at the 0.05 level (and these demonstrated *disassortative* interactions). A combined probabilities test for all experimental groups showed a close fit to random interactions (Fisher's combined probability test, d.f. = 20, $P > 0.1$). Limiting the analysis to the days on which the predator was shown did not change the results. For the control trials, one slope differed from -0.2 at the 0.05 level and a combined probabilities test for all groups showed a close fit to random interactions (Fisher's combined probability test, d.f. = 22, $P > 0.3$).

One reason why high inspectors may fail to associate preferentially with other high inspectors is that they may be unable to avoid the company of low inspectors. Under such a scenario, high inspectors might be the nearest neighbour to more fish than low inspectors, but the average inspection score of these nearest neighbours would not depart from randomness. However, no correlation was found between an individual's inspection score and the number of times it was a nearest neighbour, for either the experimental trials (adjusted $r^2 = -0.18$, $P > 0.9$) or the control trials (adjusted $r^2 = 0.034$, $P > 0.7$). Limiting the analysis to the days on which the predator was shown did not change the results (adjusted $r^2 = -0.14$, $P > 0.6$ for predator days; adjusted $r^2 = -0.007$, $P > 0.4$ for no-predator days).

DISCUSSION

Although, in principle, cooperation can evolve without assuming sophisticated animal cognition, it is more likely to evolve if animals can remember individuals with whom they have interacted and how such interactions unfolded, as such information can be useful with respect to future information. Since early work on cooperation and inspection in guppies (Dugatkin and Alfieri, 1991b) suggested a limited form of assortative interactions, once a model of assortative interactions in large groups was developed, the guppy system seemed an obvious place for empirical examination of such a model.

Dugatkin and Alfieri's (1991b) results suggest that inspectors are *capable* of preferentially interacting with more cooperative co-inspectors, but two caveats are worth noting. First, these experiments show only that guppies have the ability to distinguish between cooperators and defectors, not that they do so in natural or semi-natural environments. For

example, Dugatkin and Alfieri (1991b) used only three guppies, and fish were separated at all times by Plexiglas partitions. How guppies would behave in larger groups in a more open space was not examined. A second, related caveat is that, since cooperators and defectors both prefer to associate with cooperative inspectors, it is not clear how such preferential assortment would manifest itself in a natural environment. To address both these points, and to test the basic predictions of Wilson and Dugatkin's (1996) model, we ran the experiments described here. Contrary to our *a priori* predictions, we found no evidence of assortative interactions (with respect to cooperation) in free-swimming groups of six guppies.

Our failure to demonstrate assortative interactions obviously does not imply their absence in other species or even in guppies under other circumstances. The current status of assortative interactions as a mechanism for the evolution of cooperation is paradoxical. Theoretically, they appear more plausible than ever before. Empirically, our effort to find them in guppies has not succeeded. Only additional research will resolve this paradox.

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