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## Social structure and co-operative interactions in a wild population of guppies (*Poecilia reticulata*)

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**Abstract** In contrast to the substantial number of theoretical papers that have examined the mechanisms by which cooperation may evolve, very few studies have investigated patterns of co-operation in natural animal populations. In the current study, we use a novel approach, social network analysis, to investigate the structure of co-operative interactions in the context of predator inspection in a wild population of guppies (*Poecilia reticulata*). Female guppies showed social preferences for stable partners, fulfilling a key assumption made by models of reciprocity. In the laboratory, wild female guppies disproportionately engaged in predator inspection with others with whom they had strong social associations. Furthermore, pairs of fish that frequently engaged in predator inspection did so in a particularly co-operative way, potentially reducing costs associated with predator inspection. Taken together, these results provide evidence for assortative interactions forming the basis of co-operation during predator inspection in a natural fish population. The occurrence of highly interconnected social networks between stable partners suggests the existence of co-operation networks in free-ranging populations of the guppy.

**Keywords** Social networks · Shoaling · Tit-for-tat

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### Introduction

Understanding the evolution of co-operation has captured the imagination of scientists for over 100 years (Darwin 1859; Kropotkin 1908; Allee 1951; Hamilton 1963; Trivers 1971; Wilson 1975; Milinski 1987; Dugatkin 1997). Co-operative acts have been described across a diverse array of species and contexts. For example, international governments form co-operative security agreements to give military assistance (Long 2003); salmon fishermen in Alaska share information about the location of fishing grounds within small carefully chosen groups (Gatewood 1984); vampire bats share blood with roost mates who have failed to obtain food in the recent past (Wilkinson 1984); and chimpanzees form co-operative hunting groups (Boesch 1994). Understanding ‘who co-operates with whom’ and what factors determine patterns of co-operation are fundamental issues, spanning biology, economics, psychology, anthropology and the political sciences.

In the early 1970s, Trivers (1971) identified that co-operation could evolve between unrelated individuals via reciprocity, whereby individuals are altruistic to those who have previously been altruistic towards them (termed ‘reciprocal altruism’). The evolution of reciprocal altruism may be facilitated through assortative interactions between co-operative individuals, minimising costs due to defection (Wilson and Dugatkin 1997). However, despite the immense interest in the topic of co-operation, with notable exceptions (e.g. Wilkinson 1984), very few investigations have examined patterns of co-operative behaviour in natural animal populations.

Much of the early empirical progress on the evolution of co-operation focused on predator inspection, particularly using species of small freshwater fish (Milinski 1987; Dugatkin 1988). Predator inspection describes a behaviour whereby individuals leave the relative safety of a group to approach and inspect a predator, gaining information on the predator’s state and on the probability of attack (Pitcher et al. 1986). Experimental studies reveal that inspecting individuals are exposed to increased predation risk (Dugatkin 1992; Milinski et al. 1997); however, individuals often share

the risk by inspecting the predator in a co-operative partnership (Milinski 1987; Dugatkin 1988). Previous laboratory work has demonstrated that predator inspection fulfils several assumptions made by previous models for the evolution of co-operation via reciprocity (Wilson and Dugatkin 1997; Dugatkin and Wilson 2000). For instance, predator inspection is a reliable quantitative behavioural trait that can be observed by other members of the population. Moreover, individuals monitor the inspection tendency of others and prefer to associate with inspectors over non-inspectors (Milinski et al. 1990a; Dugatkin and Alfieri 1991). However, there has been little attempt to extend this work into the wild and investigate patterns of co-operation in the context of predator inspection in natural populations.

Recent work suggests that conditions in the wild may be favourable for the evolution of co-operation in some species of small freshwater fish (Ward et al. 2002; Croft et al. 2004b; 2005). For example, Croft et al. (2004b) constructed social networks for wild guppy (*Poecilia reticulata*) populations by connecting individuals via their social affiliations (defined as co-occurrences in the same shoal recorded over a 7-day period). Within the social networks, female guppies were observed to occur in stable social interactions fulfilling another important assumption underlying the evolution of co-operation (see Dugatkin 1997). However, the stability of such interactions over longer periods of time (i.e. more than 7 days), and the role of active choice (or avoidance) in generating the observed persistent social interactions in the wild, remains unknown. Finally, the extent to which such interactions form the basis of co-operative alliances has not been investigated.

There has been much controversy in the literature over what constitutes acts of co-operation and as to the exact mechanisms underlying co-operative acts. Resolution of this debate requires extensive investigation into the costs and benefits of co-operative behaviour. Whilst future laboratory work might usefully focus on this issue, there is a need to examine the patterns of co-operative interactions in wild animal populations to test predictions made by various models of co-operation.

This study uses social network theory to investigate the patterns of co-operation in a wild population of guppies. The potential of social network analysis in the behavioural and ecological sciences is just beginning to be explored. An understanding of the structure of a social network may allow us to make important predictions about a population (Newman 2003). For example, in the context of co-operation, information on 'who interacts with whom' and the stability of those interactions through time may make it possible to predict 'who will co-operate with whom'.

The guppy is a classic model in evolutionary and behavioural biology and as a result much is known about its behaviour and ecology (Magurran et al. 1995). Guppies typically shoal in nature (Seghers 1974) and leave the relative safety of shoals to inspect potential fish predators in a manner that is risk-sensitive (Dugatkin and Godin 1992; Magurran and Seghers 1994a).

Initially, we used mark, release and recapture techniques to evaluate social network structure in natural guppy pop-

ulations, identifying if conditions are favourable for the evolution of co-operation. Secondly, we used information on social network structure of wild caught shoals to predict patterns of co-operation, which we tested in a series of laboratory investigations of predator inspection. We predicted that, in populations under a high risk of predation, non-random partner selection based on inspection tendency would be manifested in the social network structure. In particular, we predicted that assortative interactions based on co-operation would lead to stable social interactions in the network, beyond that explained by morphological assortment. Finally, we predicted that those individuals in stable interactions would be more co-operative when engaging in predator inspection than those in unstable interactions.

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## Materials and methods

### Quantifying the structure of social networks in the wild

Initially we investigated the structure of social networks in wild populations of guppies. In particular, we explored 'Who interacts with whom?', 'How stable are these interactions through time?' and 'What is the role of behaviour in structuring these interactions?' Whilst previous work has examined the structure and stability of social interactions in guppies over short (7 days) time periods (Croft et al. 2004b), the stability of these interactions over longer time periods and the role of individual preferences in structuring such interactions remain unknown.

All adult guppies were captured from two pools (populations) in the Arima River (10° 41'N, 61° 17'W), Trinidad during May 2003. After capture, each population was kept in a separate holding arena for 24 h (diameter=160 cm, water depth=15 cm). The fish were given individual identity marks using a visible implant elastomer (VIE) injected in the dorsal epidermis (see Croft et al. 2003a for details). The fish were then simultaneously released into the centre of their original pool. Re-sampling began 24 h after release following the methodology described by Croft et al. (2004b), and was undertaken daily for a period of 15 days, and then repeated on days 20 and 31. The two extra sampling days were conducted to provide additional information on the stability of social interactions over extended time periods. During each re-sampling event, entire shoals were captured from the pools. Shoal composition was defined as fish within four body-lengths, which is well within the inter-individual distance of fish within the same shoal (Pitcher et al. 1983). Shoals were released at the point of capture once the identity of each individual in all shoals had been recorded. Shoals of guppies encounter other shoals frequently (approximately every 14 s), often exchanging individuals. They disperse overnight, resulting in the breakdown of shoal composition and a re-assembly every morning (Croft et al. 2003b). Therefore, samples made on consecutive days can reasonably be assumed to be independent.

We used the number of times individuals were observed in the same shoal as others as an indication of the strength of

the association (AS). We compared the observed number of persistent pairs [defined as those with  $AS \geq 3$ , i.e. occurred in the same shoal together three or more times (Croft et al. 2004b)] in each population to an expected value generated from a null model of shoal membership. The expected values were calculated using a constrained randomisation approach, whereby we generated computational data sets for each network containing the same number and size of groups as the observed data (see Croft et al. 2004b for details). These computational data sets were generated by reallocating marked fish at random to hypothetical groups of the observed recaptured daily shoal sizes (see Ward et al. 2002). This operation was repeated 1,000 times to provide expected frequency distributions of our test statistic (see Crowley 1992). Each randomisation of the experimental data controlled exactly for the observed shoal sizes and recapture frequencies of individual fish. *P* values were obtained by comparing the expected values for pair-wise interactions with an  $AS \geq 3$  with the observed ones.

Are repeated pair-wise interactions based on more than morphological assortment?

To test whether fish that formed stable social interactions in the network really did have a social preference for each other over other individuals in the populations beyond that explained by morphological assortment, the shoaling preferences of females in persistent pairs were investigated on day 8 of the daily shoal captures. Each trial consisted of three fish, two of which had an  $AS \geq 3$  with one designated as the ‘focal fish’, assigned at random, and the other the ‘associate’. A third fish (designated the ‘control fish’), taken from the same population and of a body length and sex matching that of the associate, was selected from those individuals rarely ( $AS < 3$ ) or never found with the focal fish in the wild. All three fish were entered at the same time into a circular arena (120 cm diameter, water depth=10 cm) at the riverside and left for 10 min to settle down. To generate semi-natural conditions, the arena contained two stones (approximately 20 cm in diameter) placed randomly. Over a period of 6 min observations were made every 15 s, recording whether the control or the associate fish were the nearest neighbour when shoaling with the focal fish. We also recorded the number of observation events the focal fish was on its own (defined as being greater than four body lengths from a second fish, see above). Observations were made directly by an observer who sat motionless 2 m from the arena. Pilot trials showed that the fish did not respond to the presence of the observer. During the trials individuals were identified using the VIE markings on their dorsal surface (see above).

Can social network structure predict patterns of predator inspection?

We investigated if social network structure of wild caught shoals of female guppies (that will have had the opportunity to develop assortative interactions under natural condi-

tions) can predict patterns of co-operation. We used female guppies, as they are responsible for the observed stable interactions in the wild (see above). Initially we quantified the structure of the social networks, which we used to predict association patterns during predator inspection.

A total of 19 shoals of guppies, ranging in size from 11 to 37 individuals including both males and females (mean  $\pm$  SD=19.7 $\pm$ 7.5), were captured from the Arima River, Trinidad between late May and early July 2004. A maximum of 12 (min=8, mean  $\pm$  SD=11.3 $\pm$ 1.08) females from each shoal were given individual identity marks using a VIE injected in the dorsal epidermis (for details see Croft et al. 2004b). The shoals of marked females were placed in an experimental arena (circular pool, diameter=120 cm, water depth=8 cm, water temperature=26°C, one per shoal), and left for an acclimatisation period of 15 h. The social network for each shoal was quantified by visually recording association patterns once per minute over a 30-min period. Previous work has shown this to be a sufficient time period to quantify non-random structure of guppy shoals (see Croft et al. 2004b online supplementary material). Observations on shoal composition were made directly by two observers who sat motionless 2 m from the arena with each observer recording the identity of approximately six fish. Pilot trials showed all 12 fish could be identified within 10 s and that the fish did not respond to the presence of the observers. An association was defined as fish within four body lengths, a distance that falls within the range of inter-individual distances most commonly observed in shoaling fishes in nature (Pitcher and Parrish 1993). The AS of pairs of individuals was calculated as the number of times they co-occurred in the same shoal over the 30 observations and was used to predict patterns of predator inspection.

Following quantification of the social network, a live cichlid fish predator, *Crenicichla frenata*, (and a natural predator of the guppies in the wild) was presented to the fish in a transparent cylinder (diameter=16 cm) placed in the centre of the arena that allowed subjects to detect visual but not olfactory cues. Initially, a visual barrier (diameter=26 cm) surrounded the cylinder, which was subsequently lifted using a remote pulley mechanism after 30 min. The composition of shoals inspecting the predator was recorded over the following 30 min period. Inspection events were defined as fish that orientated towards the predator and directly approached the predator (Dugatkin and Godin 1992; Magurran and Seghers 1994a). To standardise recording of the inspection events, we only recorded inspections that occurred within a 21-cm radius (representing approximately seven body lengths) of the predator cylinder. Pilot observations demonstrated that most inspection events occurred within this radius. To qualify as ‘in the same inspecting shoal’, two fish had to be within four body lengths of each other (see above) during the inspection event. We then calculated the number of times a pair of fish inspected a predator together (defined as the number of times they co-occurred in the same shoal during predator inspections, henceforth, ‘inspection strength’ or IS of pairs of individuals in the predator inspection network), and compared this value to the AS of pairs in the social network

using a Mantel test for matrix comparisons.  $P$  values for each shoal were then combined using Fisher's omnibus test (Haccou and Meelis 1992).

Does predator inspection show elements of co-operative behaviour?

The number of times a pair of fish inspected a predator together in the third experiment was used to predict the degree of co-operation (as defined below) in the fourth experiment. For each network we identified two pairs of fish: firstly, a pair with a high inspection strength ( $\geq 3$ ) predicted to co-operate, and secondly, a pair with a low inspection strength ( $\leq 1$ ) predicted to have low levels of co-operation. We compared the inspection behaviour of pairs with high and low inspection strengths. We controlled for overall inspection tendency by selecting fish that showed a similar inspection frequency [maximum difference between the inspection frequency = 18%, mean ( $\pm$ SD) = 7.19  $\pm$  5.5%]. The experimental aquarium (length = 76 cm, height = 30.5 cm, width = 30.5 cm) consisted of a predator chamber (a cylinder 16 cm in diameter) located at one end of the aquarium and a release chamber (length = 20 cm, height = 30.5 cm, width = 30.5 cm) located at the other end of the aquarium, formed by lowering an opaque partition. At the beginning of each trial, the test-pair was allowed to swim freely in the aquarium for 5 min in the absence of the predator and with the opaque partition of the release chamber raised. After the 5-min introduction period the fish were moved into the release chamber by herding the fish using a 7  $\times$  9 cm dip net and the opaque partition was lowered using a remote pulley mechanism. A predator (*C. frenata*) was introduced to the predator chamber. The fish were then left for another 5 min before the partition was raised, marking the start of the trial.

The beginning of a predator inspection event was defined as when the first fish moved out of the release chamber and orientated towards the predator. We recorded the degree of co-operative behaviour during the first inspection (see below for a definition), which was defined as finished when both fish orientated away from the predator. Given that fish may assess their inspection partners on the basis of body length (Kulling and Milinski 1992), we ensured that there was no significant difference in the degree of body length assortment between pairs with high and low inspection scores (median body lengths  $\pm$  inter-quartile range, high inspection scores = 25.5 mm, +2.5 mm, -2.5 mm, low inspection scores = 26.5 mm, +1.75 mm, -2.5 mm, Wilcoxon signed ranks test  $N=20$ ,  $Z=-0.76$ ,  $P=0.44$ ). All trials in the current experiment were recorded using video equipment, and subsequently analysed blind.

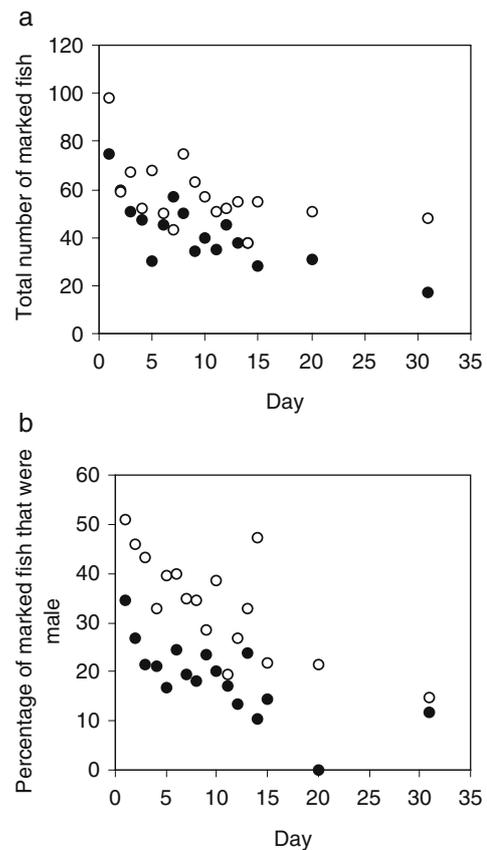
We defined acts of co-operation as instances when individuals reduced the predation risk of their inspection partner at a cost of increased risk to themselves, which we measured using a number of response variables: (1) the latency of the second fish to commence inspection following commencement by the first (where a short latency is indicative of risk-sharing and hence co-operation), (2) the maximum distance between the fish during the predator

approach (where a short distance is indicative of co-operation) and (3) the number of times individuals exchanged the lead position, (where a large number of changes of lead is indicative of co-operation). Although predation risk to any one individual can be reduced by inspecting in a pair (Milinski 1987), the remaining risk is not shared equally, and there is a disproportionate risk of predation for the lead fish (Milinski et al. 1997; Krause et al. 1998). Thus, we predict that in a co-operative pair the latency for the second fish to commence inspection will be lower, the maximum distance between inspecting fish will be smaller, and there will be more over-taking events. The latter follows from the findings of theoretical analyses exploring solutions to the iterated prisoner's dilemma, which suggest the efficacy of a 'tit-for-tat' strategy (Dugatkin 1997).

## Results

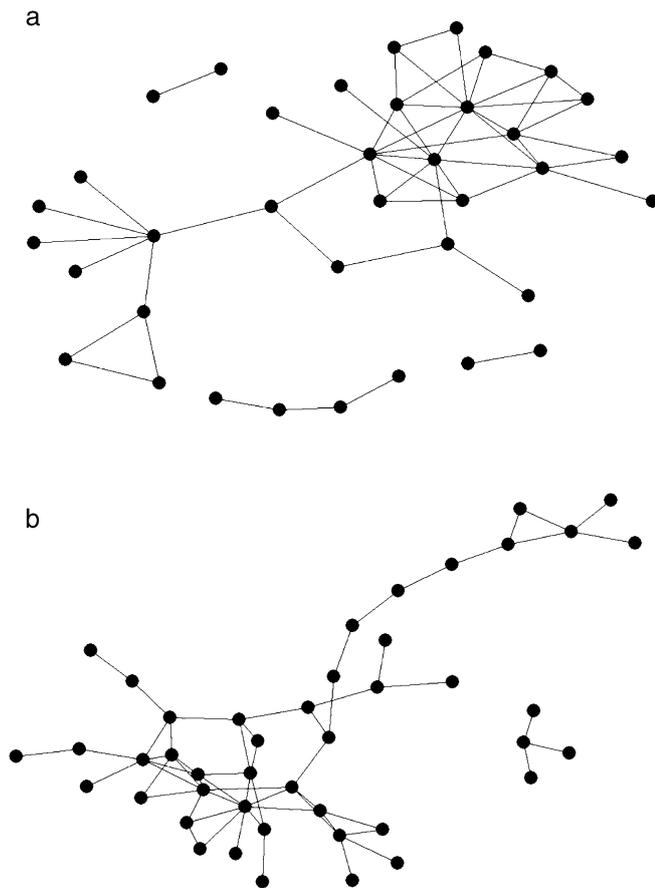
### Social network structure in the wild

A total of 110 [females = 81, males = 29, mean ( $\pm$ SD) total length = 23.9  $\pm$  4.1 mm] and 143 [females = 77, males = 66,



**Fig. 1** **a** Total number of marked fish captured in the two populations (population 1 = ●, population 2 = ○, Spearman correlation: population 1,  $n=17$ ,  $r_s=-0.76$ ,  $P<0.01$ , population 2,  $n=17$ ,  $r_s=-0.57$ ,  $P=0.02$ ) **b** The percentage of marked fish captured on a given day that were male (population 1 = ●, population 2 = ○, Spearman correlation: population 1,  $n=17$ ,  $r_s=-0.75$ ,  $P<0.01$ , population 2,  $n=17$ ,  $r_s=-0.69$ ,  $P<0.01$ )

mean ( $\pm$ SD) total length=25.17 $\pm$ 6.26 mm] adult guppies were captured marked and released in populations 1 and 2, respectively. Substantial proportions of marked fish were recaptured on day 31 of the investigation (population 1=15.45%, population 2=33.6%; Fig. 1a), but the percentage of males in the samples significantly decreased over time (Fig. 1b). Based on our daily recaptures of shoals, social networks were constructed (see Fig. 2) within which significant and persistent pair-wise interactions were observed more frequently within the populations than expected by random associations (Table 1). This result is consistent with previous observations by Croft et al. (2004b; 2005). Further analysis revealed that this pattern was largely due to female-female pair-wise interactions, which were distributed over extended time periods (mean $\pm$ SD, population 1=11 $\pm$ 7 days and population 2=14 $\pm$ 9 days).



**Fig. 2** The social networks for population 1 (a) and 2 (b) constructed from information on persistent female-female interactions in the wild ( $AS \geq 3$ ). In this network, each circle (node) represents an individual marked fish. A line (edge) connects the circles representing two fish, if during all of the daily recapture procedures, those fish were caught together in the same shoal on three or more days. The networks were drawn using the UCINET program (Borgatti et al. 2002), using the “spring embedding” algorithm to determine the layout of the nodes and edges

**Table 1** The number ( $N$ ) of pair-wise interactions ( $AS \geq 3$ ) observed in the two wild populations (mf=male–female, mm=male–male and ff=female–female pairs)

	Population 1	Population 2
All pairs	$N=55, P=0.02$	$N=75, P<0.001$
mf pairs	$N=9, P=0.4$	$N=23, P=0.14$
mm pairs	$N=1, P=0.38$	$N=3, P=0.79$
ff pairs	$N=45, P=0.02$	$N=49, P<0.001$

The table also shows the probability that these interactions occurred more frequently than expected by random assortment

Are repeated pair-wise interactions based on more than morphological assortment?

A total of five trials (consisting of five shoaling pairs and five different associate fish) were conducted. Of the time spent shoaling, focal fish spent significantly more time with the ‘associate’ than the control fish (mean $\pm$ SD proportion of observations with the associate=83.2 $\pm$ 14.26%; One Sample  $t$  test,  $t=5.2, N=5, P=0.006$ ).

Can social network structure predict patterns of co-operation?

A significant positive correlation was found between the AS of a pair-wise interaction in the social network and the IS during predator inspection (Fisher’s omnibus test  $f_{38}=77.12, P<0.001$ ).

Does predator inspection show elements of co-operative behaviour?

A significant overall effect of inspection tendency was observed on our measures of co-operation (Table 2). Further analysis revealed that this was due to a significant

**Table 2** Multivariate ANOVA of measures of co-operation during predator inspection (**latency**: the latency of the second fish to commence inspection following commencement by the first; **distance**: the maximum distance between the fish during the predator approach; **overtaking**: the number of times individuals exchanged the lead position)

Source	Mean ( $\pm$ SD)		$df$	Mean square	$F$	$P$
	IS $\leq$ 1	IS $\geq$ 3				
Within subject effects						
Inspection strength			3		3.78	0.032
Between subject effects						
Latency	15.9 $\pm$ 32.0	6.4 $\pm$ 6.9	1	451.25	0.84	0.37
Distance	10.2 $\pm$ 9.5	12.9 $\pm$ 7.5	1	37.13	0.51	0.48
Overtaking	0.7 $\pm$ 0.7	2.7 $\pm$ 1.7	1	20	11.92	0.003

effect of inspection strength on the number of overtaking events in pairs with high inspection scores (mean $\pm$ SD=2.7 $\pm$ 1.7) exchanging lead position more often than those with low inspection scores (mean $\pm$ SD=0.7 $\pm$ 0.67) (Table 2).

## Discussion

Despite its theoretical appeal, evidence for co-operation based on reciprocity in wild animal populations has been conspicuously lacking from the literature (Hammerstein 2002), but see Trivers (2004) for notable exceptions. Using a combination of both field and laboratory work incorporating social network theory, we investigated the structure of co-operative interactions in a wild fish population and observed assortative interactions of the nature predicted by models of reciprocity. Furthermore, such interactions appear to form the basis of co-operative alliances during predator inspection.

The evolution of co-operation is thought to be constrained by the probability that individuals will repeatedly encounter each other (see Dugatkin 1997). Previous laboratory work on co-operation in fish suggests that wild populations may contain many long-lasting pair-wise interactions thus overcoming this constraint (Milinski et al. 1990b; Ward et al. 2002). Here, we document the occurrence of stable interactions between female guppies in the wild. This finding is strikingly consistent with the predictions of theoretical investigations of the evolution of reciprocal co-operation, in particular, with tit-for-tat solutions to the iterated prisoner's dilemma (see Dugatkin 1997 for a review). The lack of males from persistent pair-wise interactions is probably the result of sex differences in mating strategies (see Magurran and Seghers 1994b). In male guppies, reproductive output is primarily constrained by the total number of copulations. In contrast, it is dependent on the pre-natal investment in offspring (reflecting size, body condition, and food reserves) of female guppies and in their selection of high-quality mates. As a result, males are typically more mobile than females both socially and spatially (see Croft et al. 2003a; 2003b). This potentially explains the observed decrease in the percentage of males captured as a function of time in the current investigation. Such mobility may constrain the potential for male guppies developing stable social interactions (Griffiths and Magurran 1998).

Phenotypic assortment by body length and sex is known to occur within the wild population (Croft et al. 2003b; 2004a), and this may contribute to the non-random social structure observed here (Croft et al. 2005). However, the results of our shoaling preference tests demonstrate that female guppies display a preference for their observed social partners above and beyond that explained by assortative interactions based on body length and sex. These observations suggest that individuals develop active social preferences for certain conspecifics and are consistent with the notion that individuals prefer to associate with others

that are 'familiar' (Griffiths 2003). Associations between familiar individuals may be adaptive, for example, by reducing predation risk through co-ordinated anti-predator behaviour (see Griffiths 2003; Ward and Hart 2003 for reviews). Furthermore, such assortative interactions fulfil important pre-requisites for the evolution of co-operation via reciprocity (see Dugatkin 1997).

The social network structure of wild caught female guppy shoals was found to be a good predictor of the predator inspection network, with individuals disproportionately engaging in predator inspection with others with whom they had strong social associations. Furthermore, pairs of fish that frequently engaged in predator inspection did so in a more co-operative way, with individuals in pairs with high inspection strengths exchanging lead position more often than those with low inspection strengths. Given the increased risk of predation for the lead fish when inspecting in a pair (Milinski et al. 1997; Krause et al. 1998), changes in the lead position strongly suggest an act of co-operation based on reciprocity, in a manner predicted by the 'tit-for-tat' strategy.

Whilst other mechanisms underlying co-operation could be invoked to explain our observations, we believe that reciprocity is the most plausible one. We cannot rule out kin-directed selection because the degree of relatedness between individuals was unknown. However, a recent study found no evidence for kin assortment in free-ranging shoals of guppies (Russell et al. 2004), such as those used here. Whilst it has been suggested that co-operation based on assortative interactions may be driven by group selection (Wilson and Dugatkin 1997), our findings suggest that groups at a level above the pair are too fluid for group selection to operate. Regardless of the precise nature of the mechanism of co-operation in operation, repeated interactions in the social network were a good predictor of co-operative relationships between female guppies during predator inspection.

Finally, we show that social network structure can be used to predict patterns of co-operation in a population of wild animals. For example, many females in the wild maintain more than one persistent relationship, giving rise to entire networks of persistent social interactions. Our work on social and inspection networks under semi-natural conditions shows that repeated interactions between individuals are a good indicator of co-operative relationships between female guppies. The tantalising prospect that these findings raise is that the networks of persistent associations in the wild are co-operative networks based on repeated pair-wise interactions within a population.

In summary, our experiments with Trinidadian guppies show that: (1) natural shoals of guppies are composed of stable associations between individuals, found together more persistently than chance expectation; (2) these stable associations are unlikely to be a result of morphological assortment; (3) that such associations can be used to construct social networks, which predict patterns of behaviour in predator inspection; and (4) that predator inspection behaviour is consistent with the concept of co-operation.

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