BEHAVIORAL ECOLOGY - ORIGINAL RESEARCH

The role of relatedness in structuring the social network of a wild guppy population

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Abstract The role of relatedness in structuring animal societies has attracted considerable interest. Whilst a significant number of studies have documented kin recognition in shoaling fish under laboratory conditions, there is little evidence that relatedness plays a significant role in structuring social interactions in wild populations that are characterised by fission–fusion dynamics. Previous work has tended to compare relatedness within and among entire shoals. Such an approach however, does not have the ability to detect social sub-structuring within groups, which appears to be a major factor driving the social organisation of fission–fusion animal societies. Here, we use social network analysis combined with DNA microsatellite genotyping to examine the role of relatedness in structuring social relationships in a wild population of guppies (*Poecilia reticulata*). Consistent with previous findings, female–female dyads formed the strongest social relationships, which were stable over time. Interestingly, we also observed significant co-occurrence of male–male interactions, which is in contrast to previous work. Although we observed social sub-structuring in the population, we found no evidence for relatedness playing a significant role in underpinning this structure. Indeed, only seven first-degree relative dyads were identified among the 180 fish genotyped, indicating that the majority of individuals do not have a first-degree relative in the population. The high genetic diversity observed in this population is indicative of a large effective population size typical of lowland guppy populations. We discuss our findings in the context of the evolution of social organisation and the mechanisms and constraints that may drive the observed patterns in wild populations.

Keywords Group living · Cooperation · *Poecilia reticulata* · Social organisation · Social assortment

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Introduction

Living in social groups is an adaptive behavioural strategy that can reduce predation risk and provide foraging benefits (Krause and Ruxton 2002). Whilst individuals can gain benefits by simply associating with others, the benefits of grouping can be increased by associating with individuals of a particular phenotype (Landeau and Terborgh 1986; Neill et al. 1974; Ohguchi 1978; Schradin 2000; Theodorakis 1989). For example, the anti-predator benefits of grouping can be enhanced by associating with individuals that are of the same phenotype (e.g., colour, size, sex) to reduce a predator's capture success rate due to the confusion effect (Landeau and Terborgh 1986; Ohguchi 1978; Theodorakis 1989). The adaptive benefits of grouping can be enhanced by associating with kin, which can provide indirect fitness benefits (Hamilton 1964). For example, associating with

kin can provide indirect benefits of performing costly cooperative behaviours, such as predator inspection (Milinski 1987), and reduce aggression (Olsén and JäUrvi 1997), while kin groups may show increased growth rates (Brown and Brown 1993; Brown et al. 1996; Gerlach et al. 2007).

Kin structuring is well studied in cooperative breeding societies where social groups are formed through retention of offspring (see Hatchwell 2010 for a review). However, the role of relatedness in structuring animal societies that are characterised by a dynamic fission–fusion social system remains unclear. Previous work on shoaling fish, for example, has compared estimates of relatedness within and between entire social groups (Dowling and Moore 1986; Hain and Neff 2007; Naish et al. 1993; Peuhkuri and Seppa 1998; Piyapong et al. 2011; Russell et al. 2004) and have tended not to find kin assortment, even in species that have been shown to have the capabilities for kin discrimination when tested under laboratory conditions (Fitzgerald and Morrissette 1992; Hain and Neff 2007; Van Havre and Fitzgerald 1988). One of the reasons for this discrepancy between laboratory and field studies may relate to the dynamic nature of fission–fusion social systems. Individuals may have low levels of fidelity to a particular social group and frequently move between groups. In free-ranging guppies (*Poecilia reticulata*), for example, individuals may associate with a number of different shoals over a time scale of minutes (Croft et al. 2003b). Despite these underlying dynamics, within such groups, there is mounting evidence that individuals maintain stable social relationships with particular partners and that social groups contain stable social units (Croft et al. 2004b; Klimley and Holloway 1999; Ward et al. 2002). An analysis at the level of the group does not have the resolution to detect such population social sub-structuring.

Increasingly, social network analysis is being used to quantify the social structure of wild animal populations (see Krause et al. 2007; Sih et al. 2009 for reviews). The advantage of this approach is that it integrates information on patterns of social interactions over multiple sampling events, allowing not just the occurrence of social associations to be quantified but also the strength of those associations (Croft et al. 2008). Such an approach can reveal a cryptic social structure that is not apparent using a group-based approach; as has been shown for behavioural structuring in a social network of a wild population of guppies (Croft et al. 2009b).

Here, we investigate the role of relatedness in structuring the social network of a wild population of guppies living under high predation risk. Predation risk is an important ecological variable driving kin assortment and associating with relatives can provide indirect fitness benefits to individuals when engaging in risky antipredator behaviours such as predator inspection behaviour (Dugatkin 1988;

Milinski 1987). Previous work has shown that social networks of wild populations of guppies differ significantly from those expected with random interactions; that is, individuals establish stable social relationships based on active partner choice, which results in stable social communities (Croft et al. 2006). Crucially, guppies are capable of kin recognition (Hain and Neff 2007) and recent laboratory work suggests that they prefer to associate with kin over non-kin (Evans and Kelley 2008; Hain and Neff 2007). Guppy life history provides the opportunity for the formation of social relationships structured by relatedness; females give birth to juvenile guppies in broods and there is no pelagic dispersal (Magurran 2005). Recent work has shown that shoals of juvenile guppies in the wild are assorted by relatedness (Piyapong et al. 2011). The role of relatedness in structuring adult guppy societies, however, remains unclear, and previous work has not found assortment by relatedness at the level of the shoal (Hain and Neff 2007; Russell et al. 2004). Given the dynamic nature of shoal composition in adult guppies, we may not expect to see kin assortment at the level of the shoal, as at any given point in time shoals contain a mix of chance or random interactions and stable social associations. We use DNA microsatellite genotyping and a social networks approach to examine animals that have socially interacted, to probe the strength of those social interactions and to establish the role of relatedness in driving these interactions. We predict that individuals will form stronger associations with kin over non-kin. Furthermore, given the benefits to male dispersal in guppies (Croft et al. 2003a), we predict that relatedness will play the strongest role in structuring female–female social relationships.

Materials and methods

Study population

Guppies ($n = 268$, males = 147, females = 117, sexually immature = 12) were captured from a 40-m section of the Quare River (10°40′N, 61°12′W) in the Northern Mountain Range of Trinidad in May 2009. Guppies in this location experience high predation risk from predatory fish, principally the pike cichlid, *Crenicichla alta*. Fish were collected using a 2-m seine (Croft et al. 2004b) and during sampling entire shoals (defined as two or more fish observed within four body lengths) were captured (mean ± SD shoal size = 6.8 + 4.2). This distance is based on the elective group size concept (Pitcher et al. 1983) and is a standard measure used to define shoaling behaviour in fish including guppies (Croft et al. 2003b). Following capture, shoals were isolated and transported to the laboratory in 2-L plastic storage bags. All adult fish were anaesthetized (MS-222;

Sigma Chemical) and given individual identity marks by injecting different colours of visible implant elastomer (VIE) in three of four positions on the dorsal area (Croft et al. 2003a). The identification mark does not have an effect on shoal choice behaviour (Croft et al. 2004b) or predation risk (Reznick et al. 1996). The sex and standard length of each fish was recorded. A small non-destructive fin clipping was taken from the caudal fin of each individual and stored in ethanol for molecular analysis to estimate relatedness among individuals (see below). Following tagging, fish were housed in four aquaria (l × h × w = 76 × 46 × 46 cm, water depth = 35 cm) that had natural substrate collected from the Quare River. Fish were distributed among the tanks in such a way that tanks contained multiple shoals of fish. In this way, individuals were able to maintain associations within a shoal during tank housing.

Recording social associations

Approximately 48 h after capture, all individuals were simultaneously released into the centre of the pool of capture in the Quare River. Re-sampling of the population began 24 h after release and was undertaken once per day between 1000 and 1400 hours for 12 consecutive days. Entire shoals were captured from the pools using a 2-m seine as in the initial capture of the study population. The seine net was used to constrain the fish which were then encouraged to swim into a 2-L container which was used as a photograph chamber to record group composition using a Nikon D40x digital camera. Following photographing, each shoal was transferred to a 2-L plastic storage bag, and released back to their capture location after the composition of all shoals had been recorded. Using this method, the fish were not removed from water or handled in anyway. For each sampling day (days 0 to 12), fish were defined as associating if they were observed in the same shoal.

Molecular analysis

To determine patterns of relatedness, we conducted molecular analysis on DNA extracted from a fin clip collected non-destructively from each individual. To ensure that we had the maximum amount of information on social interactions, we restricted our molecular analysis to all individuals captured on the final day of sampling ($n = 180$). Of these 180 samples, the labels were damaged in transport for 9 samples which prevented accurate reading of the individual identification labels. All 180 samples were used in estimates of relatedness; however, the social analysis was restricted to individuals that had known DNA samples ($n = 171$). Guppies were genotyped using 11 published microsatellite loci, all of which are highly variable in wild guppy populations (Table 1). DNA was extracted using the

Chelex method (Estoup et al. 1996), and microsatellite loci were amplified in a series of two polymerase chain reaction (PCR) multiplexes, with a further locus (Pr171) amplified by itself. The Type-it Microsatellite PCR Kit (Qiagen) and the following 'touchdown' PCR program were used for all reactions, 95 °C for 5 min followed by 37 cycles of 95 °C for 30 s, annealing temperature for 90 s and 72 °C for 3 min. Annealing temperatures were 3 cycles at 62 °C, 4 cycles at 58 °C, 5 cycles at 55 °C, 10 cycles at 53 °C, 5 cycles at 51 °C, 5 cycles at 49 °C and 5 cycles at 47 °C, followed by a final extension of 72 °C for 10 min and 60 °C for 35 min. Amplicons were run on two lanes on a Beckman Coulter DNA sequencer; Pr171 amplicons were pooled with those from PCR multiplex 1 (Table 1). Microsatellite genotypes were determined using the Fragment analysis on CEQ 8000 (Beckman Coulter). Observed and expected heterozygosity were calculated using GenAlEx (Peakall and Smouse 2006) and deviations from Hardy–Weinberg (HW) expectations and linkage between the microsatellite loci were investigated using GENEPOP version 4.0.10 (Raymond and Rousset 1995; Rousset 2008), with critical levels of significance for simultaneous tests adjusted using the sequential Bonferroni procedure adjusting for the number of tests (Rice 1989). Pr15 significantly deviated from HW equilibrium, but not after Bonferroni correction, whereas Pre8 and G183 significantly deviated after correction so were excluded from subsequent relatedness calculations. Thus, relatedness was calculated using nine microsatellite loci. There was significant linkage between G43 and Pr80 after Bonferroni correction. The presence and frequency of null alleles, which can affect relatedness estimates, were calculated using Microchecker (Van Oosterhout et al. 2004). Null alleles were detected in G43 and Pr39 (Table 1). Maximum-likelihood estimators of pair-wise coefficients relatedness (r), which are usually more accurate than other estimators (Milligan 2003), were calculated using ML-Relate (Kalinowski et al. 2006), using adjustment to accommodate the presence of null alleles. Sibship analysis was conducted using COLONY version 2.0 (Jones and Wang 2009), using a typing error rate set at 0.01. This approach considers the likelihood of the entire pedigree, as opposed to relatedness of individuals on a pairwise basis. However, it has limited power in differentiating between parent–offspring and full sibling relationships. In this study, it is possible that within any one sample some individuals are parents of others, as there may have been several cohorts in the same sample.

Social network analysis: the stability of social ties

To construct the population social network, observed social associations were accumulated over the 12 sampling days. A social network consists of nodes (individuals in this case)

Table 1 Microsatellite loci used in this study

Locus code	PCR multiplex	Primer fluorescent label[a]	Size range	Observed heterozygosity (HO)	Expected heterozygosity (HE)	P value for Hardy–Weinberg test	References
G43[b]	1	D2	248–352	0.806	0.872	0.1859	Shen et al. (2007)
G183	2	D3	243–327	0.793	0.847	0.0007[d]	Shen et al. (2007)
G289	1	D3	278–326	0.928	0.885	0.6694	Shen et al. (2007)
Pr39[c]	1	D4	156–192	0.670	0.643	0.8103	Becher et al. (2002)
Pr80	1	D2	141–295	0.899	0.925	0.3479	Becher et al. (2002)
Pr92	1	D3	157–177	0.698	0.675	0.1009	Becher et al. (2002)
Pr171	3	D4	221–383	0.743	0.763	0.6799	Becher et al. (2002)
Pre8	2	D4	148–344	0.506	0.906	>0.000[d]	Paterson et al. (2005)
Pre15	2	D2	193–321	0.877	0.926	0.0465	Paterson et al. (2005)
Pre26	2	D3	142–278	0.889	0.913	0.0605	Paterson et al. (2005)
Pret69	2	D4	110–214	0.839	0.842	0.4749	Watanabe et al. (2003)

[a] Beckman Coulter code

[b] Null allele frequency = 0.041

[c] Null allele frequency = 0.026

[d] Significant after Bonferroni correction

and edges (lines connecting the nodes) (see Croft et al. 2008). The strength of the edge in the social network was weighted by the number of times the dyad was observed in the same shoal. To investigate the stability of social interactions, and thus the opportunity for relationships based on relatedness to form, we examined if network edges occurred with a greater strength than expected by chance. To do this, we filtered the network at different edge weights ($\geq 2, 3, 4$ and 5) allowing us to explore the significance of social relationships at different edge strengths. At each filtering threshold, we compared the observed number of network edges to a distribution of expected values from a null model of shoal membership. To generate these expected values, marked fish were reallocated at random to the observed daily recaptured shoal sizes (see Ward et al. 2002) and the permuted data were used to reconstruct social networks. This operation was repeated 1,000 times to provide expected frequency distributions of our test statistic. P values were obtained by comparing the expected number of network edges at the given filtering threshold to the observed ones (Crowley 1992). We subdivided our analysis to examine the significance of female–female, male–male and male–female edges.

Social network analysis: the role of relatedness in structuring the social network

To quantify the role of relatedness in structuring the observed social network, we examined the relationship between relatedness and the frequency with which edges occurred in the network. To allow us to look at individuals that had repeated interactions whist maximising the sample size, we focused our analysis on network edges with an edge strength of two or more. These observed edges were subdivided into five relatedness bands ($r = 0, 0 < r < 0.088, 0.088 < r < 0.176, 0.176 < r < 0.354$ and $r > 0.354$) and we counted the number of edges that occurred within each band. Band boundaries were picked to be midway between integer powers of 0.5 as these values represent full siblings, half siblings, etc. This should make it more likely that full siblings and half siblings, etc. are allocated to different bands. For each relatedness band, we compared the observed number of network edges to a distribution of expected values from a null model of shoal membership using the same methods outlined above. We subdivided our analysis to examine the role of relatedness in structuring female–female, male–male and male–female edges. Given that the observed and expected number of edges will differ among the relatedness bands for each relatedness band, we calculated the effect size by dividing the observed number of edges by the median expected number. We compared this effect size across bands predicting that the effect size will increase as relatedness increases. In order to make our results comparable to other studies on the kin structure of fish shoals, we undertook a further analysis at the level of the shoal. We did this by calculating the mean pair-wise relatedness observed within shoals and compared this to the expected mean shoal relatedness assuming random interactions among individuals using a null model of shoal membership as outlined above.
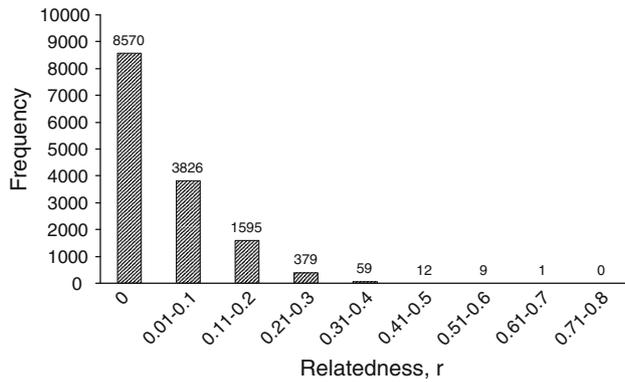
Fig. 1 Frequency distribution of pair-wise relatedness estimates (*r*) for all individuals in the study population of wild guppies (*Poecilia reticulata*) that were genotyped (*n* = 180)

Results

Molecular analysis

There was high genetic diversity in the population studied, with average expected heterozygosities for each locus ranging from 0.643 to 0.926 (average = 0.836) (Table 1). Pair-wise relatedness estimates for the 180 individuals indicated that most pairs were distantly related to each other; mean *r* between pairs was 0.036, with values ranging from 0 to 0.621 (Fig. 1). Siblingship analysis indicated that the frequency of sibling dyads was 0.022 (number of pair-wise siblingships/number of pair-wise comparisons) and seven full sibling families (which may in fact be parent–offspring pairs) were identified, each consisting of two individuals, which was the largest family size. Thus, the majority of individuals were inferred to have no first-degree relatives among the fish genotyped. A total of 352 half sibling dyads were identified, with each fish having an average of 3.91 half siblings among the 180 genotyped fish.

Social network analysis: the stability of social ties

Individuals were recaptured on average (+SD) 4.57 + 3.81 times over the 12 sampling days. The average (+SD) shoal size in which individuals were recaptured was 8.5 + 6.7. The population social network for the genotyped fish (*n* = 171) was highly interconnected and all individuals could be interconnected into a single network. Across all individuals, we observed significantly more network edges at all filtering strengths (Fig. 2a). Further analysis revealed that this pattern was largely driven by female–female social ties which occurred more frequently than expected by random interactions at all filtering strengths (Fig. 2b). Males in contrast did not form as strong dyadic interactions, and only edges with a strength ≥2 and 3 were significantly
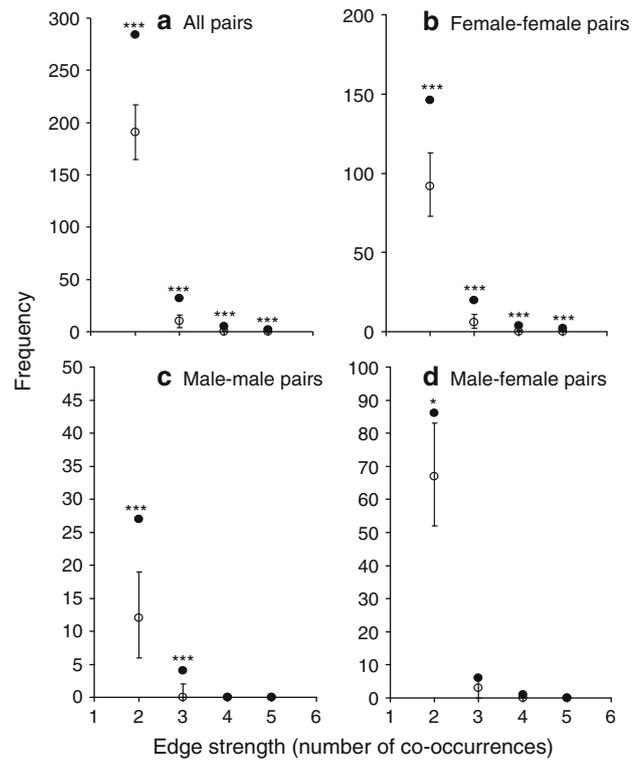

Fig. 2 Frequency of the co-occurrence of network edges in the social network of a population of wild guppies at different edge strengths (number of co-occurrences). **a** All pairs, **b** female–female pairs, **c** male–female pairs and **d** male–female pairs. Observed values (*black fill*) and expected median from the null model (*white fill*) are shown (see text for details of null model). *Error bars* represent the 2.5–97.5th percentile range. Significant differences between observed and expected frequencies are indicated by *asterisks*, ***P < 0.001 and *P < 0.05

different from that expected via random interactions (Fig. 2c). The co-occurrence of male–female edges was only significantly different from random at an edge strength ≥2 (Fig. 2d).

Social network analysis: the role of relatedness in structuring the social network

The mean relatedness of edges in the network filtered to an edge strength of two or more was 0.03. Network edges were most frequent amongst unrelated individuals (Fig. 3a–d). Across all individuals, we observed significantly more network edges in the lowest (*r* = 0 and 0 < *r* < 0.088) and highest (*r* > 0.354) relatedness bands (Fig. 3a). When we look within a sex, we see that edges only occur more frequently than we would expect by chance among unrelated individuals (females Fig. 3b, males Fig. 3c) or only very distantly related individuals (females Fig. 3b). Network edges did not occur more frequently than expected by chance among male–female dyads within any of the relatedness bands (Fig. 3d). For all individuals (Fig. 3a), we
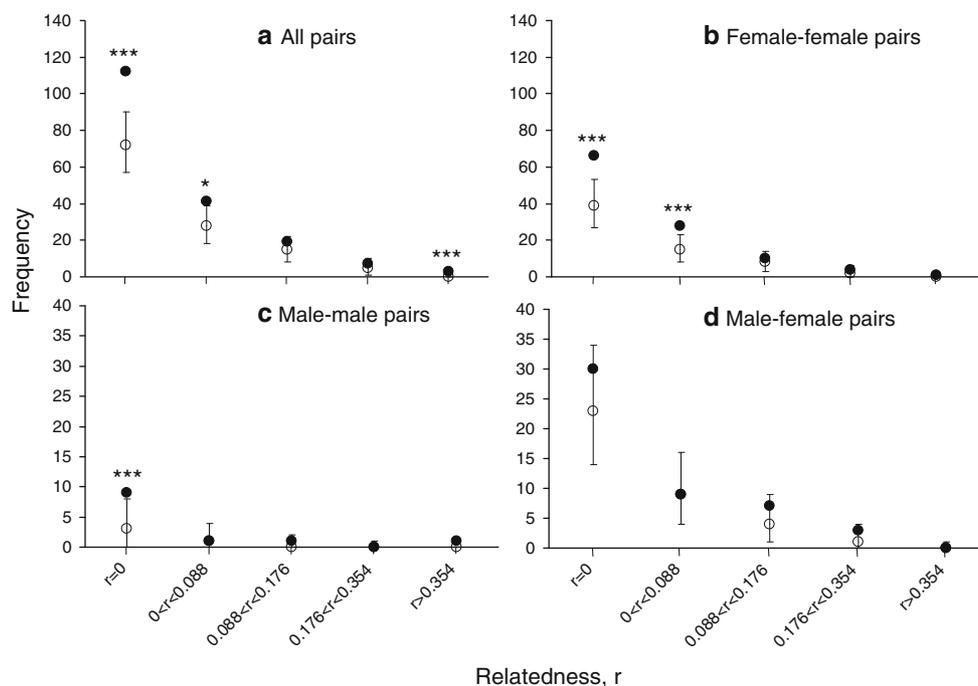
Fig. 3 Frequency of edges in the network (filtered to an edge strength of ≥2) at different strengths of relatedness. **a** All pairs, **b** female–female pairs, **c** male–male pairs and **d** male–female pairs. Observed values (*black fill*) and expected median from the null model (*white fill*) are shown (see text for details of null model). *Error bars* represent the 2.5–97.5th percentile range. Significant differences between observed and expected frequencies are indicated by *asterisks*, ***$P < 0.001$ and *$P < 0.05$
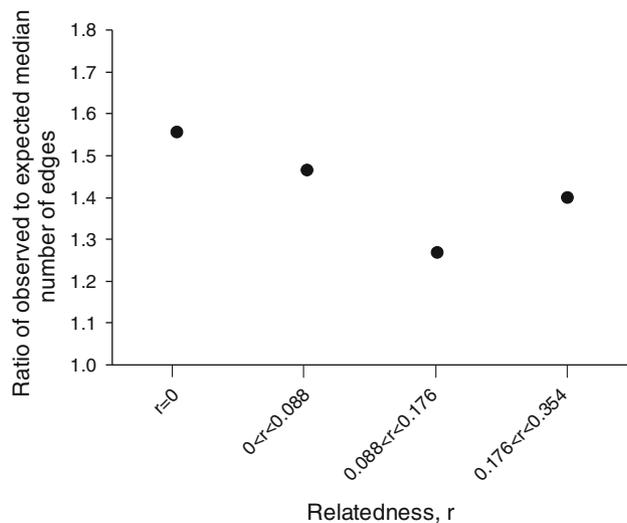

Fig. 4 Observed number of network edges divided by the median expected number at different strengths of relatedness

explored the relative role of relatedness in structuring network edges (Fig. 4). Given we only observed three edges with a relatedness value of $r > 0.354$, we restrict this analysis to the relatedness bands below $r > 0.354$ as we do not have the test power at this upper threshold to detect patterns of association. There was no evidence for an increase in effect size with increasing strength of relatedness (Fig. 4).

We performed the shoal level analysis of relatedness on shoals captured on day 4 ($n = 38$) which contained the largest number of fish captured within shoals ($n = 110$). The mean (± 1 SD) relatedness within shoals was 0.0177 (± 0.005) which did not differ from the expected mean relatedness assuming random interactions among individuals (expected mean relatedness = 0.0183, $P = 0.412$).

Discussion

Although group composition is very dynamic in guppies, our results indicated that individuals formed stable pair-wise social associations. These stable social interactions were strongest between female–female pairs, although male–male pairs did co-occur more often than expected with random assortment. Nevertheless, most pairs of individuals were distantly related to each other in this system, so the probability of encountering a related individual will be low. Whilst we find strong evidence of social sub-structuring in the study population, most social interactions occurred between unrelated individuals and the population social network was not significantly assorted by relatedness.

The high level of genetic diversity observed in the study site is typical of lowland populations, which are considerably more genetically diverse than upland guppy populations (Barson et al. 2009). High levels genetic diversity in

lowland populations appear to be maintained by ongoing migration both within and among rivers and high effective population sizes (N_e); indeed, in a previous study, the mean N_e for lowland sites was 910 (Barson et al. 2009). The guppy population examined in this study was interconnected to other pools via riffles, so guppies were able to migrate up and downstream, although most gene flow tends to be downstream (Barson et al. 2009). Values for average relatedness and the average frequency of sibship were similar to published estimates for wild juveniles (Piyapong et al. 2011). The small brood size and multiple paternity in this species may also partially explain the low number of full-sibling families identified in this study, and the low number of individuals in those families.

The observed social sub-structuring in the population is consistent with previous findings in guppies (Croft et al. 2004b) and other fish species (Klimley and Holloway 1999; Ward et al. 2002). One mechanism that may contribute to the observed co-occurrence of pairs of individuals is phenotypic assortment by morphological traits such as body size (Croft et al. 2009a). As outlined above, such assortment is known to provide adaptive benefits particularly in the context of reduced predation risk (see Krause and Ruxton 2002 for a review). Against this background of social structuring, there is strong evidence in the literature that individuals have preferred and avoided social associations based on active partner choice (reviewed in Griffiths 2003; Ward and Hart 2003), which may also contribute to the observed sub-structuring. Social recognition is adaptive: for example, it allows individuals to avoid others to whom they are competitively inferior (Metcalfe and Thomson 1995) and to develop co-operative relationships (Croft et al. 2006; Milinski et al. 1990). Associating with familiars can also reduce aggression (Utne-Palm and Hart 2000), increase foraging efficiency (Utne-Palm and Hart 2000), increase co-ordination during antipredator behaviour (Chivers et al. 1995) and enhance social learning (Swaney et al. 2001).

Given our knowledge of the guppy mating system, it is not surprising that we do not find strong stable social pairwise relationships between males and females. Reproductive success in female guppies is dependent on their selection of high quality males and investment in the offspring (Magurran and Seghers 1994). In contrast, a male's reproductive success is largely determined by his access to females and more precisely the number of successful copulations (Magurran and Seghers 1994). Males that move among shoals will thus gain a selective advantage as males show a preference for courting novel females (Kelley et al. 1999); a phenomenon that is reinforced by females being more receptive to mating attempts by novel males (Hughes et al. 1999). In contrast to previous work on wild populations of guppies (e.g. Croft et al. 2004b), we found male–male interactions significantly more often than expected if interactions were random. Previous laboratory work has shown that males can develop familiarity and prefer to associate with familiar over unfamiliar males (Croft et al. 2004a). When taken together with the current findings, the results suggest that there may be differences among populations in the benefits of and constraints on forming stable social relationships among males. Future work comparing social network structure among populations (especially those that occupy different ecological environments) would be particularly rewarding.

Our results strongly suggest that relatedness is not important in driving the structure of social relationships between adult guppies in our study population. The observed lack of kin assortment is consistent with previous work at the shoal level which includes analysis on the same high predation population used in the current investigation (Russell et al. 2004). However, in contrast to previous work (e.g. Russell et al. 2004), our approach provides the first insight into the role of relatedness in structuring adult social relationships within and among shoals. Whilst associating with kin can provide inclusive fitness benefits, when resources are limited, competition among kin can counteract kin selection for altruism (Platt and Bever 2009; West et al. 2001, 2002). In guppies, population density and resource availability differ among populations, which can drive population differences in competition (Grether et al. 2001). However, in high predation populations, such as the Quare River, competition tends to be low due to sparse rainforest canopy cover allowing for relatively high levels of primary productivity (Grether et al. 2001). Thus, it is unlikely that avoidance of competition is a major driver of the lack of kin structuring observed here. In contrast, there may be constraints on the ability of individuals to maintain kin-structured associations. For example, recent work in females has shown that sexual harassment from males can disrupt female social relationships leading to a breakdown of social network structure (Darden et al. 2009). In the wild, female guppies experience high levels of sexual harassment from males (Magurran and Seghers 1994), which may impact on their ability to maintain long-term associations with kin. Future work examining the mechanisms and constraints driving the lack of kin assortment in adult guppies would be a worthwhile research area.

Our results are particularly interesting in the context of the evolution and maintenance of cooperation in animal societies. Guppies cooperate during predator inspection, a behaviour whereby individuals leave the relative safety of a shoal to approach and inspect a predator, gaining information on the predator's state and on the probability of attack (Pitcher et al. 1986). This information is transmitted to non-inspecting individuals and provides adaptive benefits to all shoal members (Godin and Davis 1995; Magurran and Higham 1988). Inspectors pay a personal cost of increased risk

of predation (Dugatkin 1992; Milinski et al. 1997), which they can reduce by inspecting in cooperative partnerships (Dugatkin 1988; Milinski 1987). The results presented here suggest that cooperation among adult fish is very unlikely to be explained via kin selection in wild guppy populations. Explaining the evolution and maintenance of cooperation among non-kin is problematic and has received copious attention amongst theoreticians (see Fletcher and Doebeli 2009; Nowak et al. 2010 for reviews). A key to unlocking the paradox of cooperation among non-kin is an understanding of the patterns of social mixing in populations (Fletcher and Doebeli 2009; Nowak et al. 2010). In fact, recent work suggests social networks in guppies may be structured by the propensity of individuals to cooperate (Croft et al. 2009b), which could lead to the maintenance of cooperation in the absence of kin assortment (Fletcher and Doebeli 2009).

In conclusion, we studied kin structuring in a classic model system in ecology and evolution. We used a social network approach to probe the role of relatedness in structuring social relationships within and between shoals. Whilst we found significant non-random social structure, with females in particular forming stable partnerships, we found no evidence that relatedness played a significant role in driving this structure in the population studied. Taken together with previous findings, our results suggest that the lack of kin assortment is a general pattern in wild populations of guppies. However, it is important to recognise that our study was focused on a single population from an area of high predation risk. Future work comparing the role of relatedness in structuring social interactions across multiple populations of adult guppies that inhabit different ecological conditions and/or of populations that differ in size and overall relatedness would allow robust generalisations to be made.

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