MOLECULAR ECOLOGY

Molecular Ecology (2010) **19**, 810–818 doi: 10.1111/j.1365-294X.2010.04523.x

Relatedness and genetic structure in a socially polymorphic population of the spider *Anelosimus studiosus*

SARAH I. DUNCAN, SUSAN E. RIECHERT, BENJAMIN M. FITZPATRICK and JAMES A. FORDYCE

Department of Ecology and Evolutionary Biology, University of Tennessee, 569 Dabney Hall, Knoxville, TN 37996, USA

Abstract

The evolution of sociality remains a challenge in evolutionary biology and a central question is whether association between kin is a critical factor favouring the evolution of cooperation. This study examines genetic structure of *Anelosimus studiosus*, a spider exhibiting polymorphic social behaviour. Two phenotypes have been identified: an 'asocial' phenotype with solitary female nests and a 'social' phenotype with multi-female/communal nests. To address the questions of whether these phenotypes are differentiated populations and whether cooperative individuals are closely related, we used microsatellites to analyse individuals from both communal and solitary nests. We found no evidence of differentiation between social and solitary samples, implying high rates of interbreeding. This is consistent with the hypothesis that these phenotypes coexist as a behavioural polymorphism within populations. Pairwise relatedness coefficients were used to test whether cooperating individuals are more closely related than expected by chance. Pairwise relatedness of females sharing communal webs averaged 0.25, the level expected for half-siblings and significantly more closely related than random pairs from the population. Solitary females collected at similar distances to the communal spider pairs were also more closely related than expected by chance (mean relatedness = 0.18), but less related than social pairs. These results imply that low dispersal contributes to increase likelihood of interaction between kin, but relatedness between social pairs is not explained by spatial structure alone. We propose that these phenotypes represent stages in the evolution of sociality, where viscous population structure creates opportunities for kin selection and cooperation is favoured under certain environmental conditions.

Keywords: *Anelosimus studiosus*, genetic structure, microsatellites, kin selection, pairwise relatedness, spider sociality

Received 9 June 2009; revision received 1 December 2009; accepted 7 December 2009

Introduction

The fitness of any organism depends on its ability to procure resources, defend itself and reproduce. Generally, for communal living to be favoured by natural selection, the social group should meet the above requirements for an individual on average more successfully than the individual could achieve on its own (Vucetich *et al.* 2004; Whitehouse & Lubin 2005; Scantlebury *et al.* 2006; Safi & Kerth 2007; Smith *et al.* 2007). This can be achieved through cooperation, where each individual expects to benefit from the group or altruism, where some individuals make sacrifices that enhance the mean fitness of the group (Futuyma 2009, p. 417). Altruism is unlikely to evolve unless altruistic behaviours preferentially benefit individuals also possessing genes causing altruism, e.g. when interactions are between closely related individuals (Hamilton 1964; Baglione *et al.* 2003; Ebensperger *et al.* 2004). Considerable controversy persists as to whether kin selection is critical for the evolution of sociality (Wilson &

Correspondence: Sarah Duncan, Fax: +1 865 974 9461;
E-mail: sduncan7@utk.edu

Hölldobler 2005; Foster *et al.* 2006; Wilson & Wilson 2007; Hughes *et al.* 2008; Shavit & Millstein 2008). In order to address the problem empirically, studies must focus on taxa in the early transitional stages of sociality because lineages with highly elaborated systems of cooperation might have lost the signatures of initially important processes (Schwarz *et al.* 2007; Hughes *et al.* 2008).

It has become apparent that the conditions for the evolution of sociality are more complex than previously thought with more flexibility of individual roles in social structures (Schwarz *et al.* 2007). Past studies have relied on direct observation of matings, births and dispersal to estimate relatedness within populations (Roeloffs & Riechert 1988), which are difficult to obtain in wild populations. Highly variable molecular markers provide researchers the ability to estimate genetic relatedness between individuals in wild populations with unknown pedigrees, which is extremely useful in the study of the mechanisms of kin selection (Blouin *et al.* 1996; Csilléry *et al.* 2006). The interplay between cooperation and conflict require knowledge of kin composition (Ross 2001) so in systems where sociality is polymorphic and rare (e.g. spiders), the use of molecular markers allows insight into how sociality has evolved and is being maintained.

Genetic relatedness studies of the tropical social (i.e. cooperative) spider species studied to date by allozyme electrophoresis provide within colony relatedness estimated from F_{ST} and F_{IT} on the order of 0.5 to over 0.9 (Riechert & Roeloffs 1993; Smith & Hagen 1996) with higher estimates found with AFLP fingerprinting for example, 0.58 averaged over sites of *Stegodyphus dumicola* (Smith *et al.* 2009), and estimates ranging up to 0.99 (reviewed in Aviles 1997; Lubin & Bilde 2007) based on F_{ST} and F_{IT} values. Although estimates are difficult to compare across different types of data and estimation procedures, the relatedness estimates are consistent with the expected proportion of genes shared by full sibs or parents and offspring. Relatedness estimates greater than 0.5 are caused by several generations of inbreeding within colonies. Subsocial spider species (species that have extended maternal care but live solitarily as adults) examined to date with allozyme electrophoresis have a relatedness of about 0.25 within local groups (Johannesen *et al.* 1998; Johannesen & Lubin 2001), which is the proportion of genes half-siblings share in common. Thus, there might be a relationship between the degree of sociality and the level of kinship within groups. However, molecular studies have shown relatedness values in some eusocial species of ants, wasps, mole rats and sweat bees to be much lower than expected (e.g. Strassman *et al.* 1994; Burland *et al.* 2002; Kümmerli & Keller 2007; Soro *et al.* 2009).

Anelosimus studiosus

The genus *Anelosimus* Simon 1891 belongs to the comb-footed spider family, Theridiidae, that also includes the medically important widow genus, *Lactrodectus* and the cosmopolitan *Achaearanea*. There are 53 described species of *Anelosimus* (Agnarsson *et al.* 2006), including several social species; most are tropical or subtropical in distribution (Aviles 1997; Agnarsson *et al.* 2006).

Anelosimus studiosus has a subtropical to temperate distribution in North and South America. From studies completed on a population in south Florida, Brach (1977) concluded that this species is subsocial in that it exhibits extended maternal care, but defends its nest against intrusion by other females. Furey (1998) first identified multiple female colonies in this species in East Tennessee. Since Furey's (1998) initial study, the social polymorphism has been observed between latitudes and between cold and warm water environments within latitudes (Jones *et al.* 2007; Riechert & Jones 2008). While both solitary and social behavioural phenotypes are exhibited at all latitudes ranging from south Florida (26º latitude) to Tennessee (36º latitude), the social phenotype is rare at lower latitudes. The *A. studiosus* system, thus, differs from the other social spider species that are restricted to tropical and subtropical areas: *A. studiosus* exhibits a reverse latitudinal trend with more social nests at higher latitudes than in lower latitudes (Riechert & Jones 2008). The number of females per nest also increases with latitude ranging from a maximum of four to 40 females per nest at higher latitudes (see Riechert & Jones 2008).

Jones *et al.* (2007) present a model illustrating how multi-female nests might exist as a bet hedge against a mother's dying before her brood reaches independence in colder environments where juvenile development is delayed. Further study has produced evidence consistent with the brood fostering hypothesis (Jones & Riechert 2008; Riechert & Jones 2008). The model is one of cooperation, rather than altruism and does not require that cooperating females be related. However, it assumes no cost of fostering (only a fixed cost of sharing a web with a number of other females), such that cheaters or brood parasites have no detrimental effects on colony success so long as one social female survives. If such costs exist, surviving females might increase the inclusive fitness of cooperative alleles if they preferentially foster related over unrelated offspring. Even in the absence of costs, an allele that causes females to favour related over unrelated offspring would tend to spread in a population where fostering is indiscriminant.

In this study, the genetic relatedness among individuals of a mixed phenotype population of *A. studiosus* is

examined at (36º N latitude), where multi-female nests approach a frequency of 15% in a predominantly solitary-female nest system. We applied five microsatellite markers to test for genetic differentiation between phenotypes, to test the hypothesis that multi-female colony members are more closely related than are solitary individuals living at similar spatial distances from one another and whether in both situations, pairs of females are more closely related than expected from random pairings of individuals.

Materials and methods

Data collection

We established two transects (100 m each) through an *A. studiosus* population that contained mixed single female and multi-female nests at a 36º latitude site in east Tennessee (Hardin Valley Park at the junction of Hickory Creek with Melton Hill Lake, Knox County, TN, USA). We marked every 5-m interval with flagging to permit us to collect pairs of spiders from meter intervals dictated by random numbers generated in Microsoft Excel. We searched an interval for a multi-female nest and on finding one, measured its widest breadth (greatest potential between-individual distance) before collecting a pair of adult females from it. If no social nest was found at a given location, the next random location was visited. Using the same protocol, we located a pair of single female nests matching the inter-individual distance recorded for the pair of females collected from the multi-female nest. We placed each female in 70% ethanol in an individually labelled plastic vial for subsequent molecular analyses of pairwise genetic relatedness. A total 50 sets of multi-female and solitary-female nest pairs were collected (200 spiders). This sampling design provided 50 independent pairwise comparisons of relatedness between multi-female and solitary female nests. Potential population viscosity can confound the effects of individual relatedness associated with phenotype. The sampling design was developed to be able to partition the effects of inter-individual distance from phenotype. The communal nests have a low frequency of representation relative to the solitary nests and this limits the sample sizes obtainable in the local population.

To validate the usefulness of the markers, we also scored nine parent/offspring and sibling pairs of *A. studiosus* from a lab colony. The lab colony was established from spiders collected from an east Tennessee site. The average pairwise relatedness calculated for the parent/offspring and sibling pairs was 0.45, which is close to the expected value of 0.5 for parent to offspring and sibling relationships. Therefore, the markers used were assumed useful for the pairwise relatedness estimates in this study.

DNA extraction and molecular analysis

Each *A. studiosus* adult female was ground in a 1.5 mL microcentrifuge tube in 180 μL lysis buffer ATL and 20 μL of proteinase K. The mixture was incubated for 3 h or overnight at 55 °C. DNA was extracted according to standard protocol of the Qiagen DNeasy blood and animal tissue extraction kit.

We obtained a library and microsatellite primers for *A. studiosus* from Genetic Identifications Services (http://www.genetic-id-services.com). Microsatellites or simple sequence repeats, are tandemly repeated motifs of 1–6 bases found in all genomes analysed to date (Zane *et al.* 2002). Microsatellites are among the most variable types of DNA sequence in the genome and are extensively polymorphic (Ellegren 2004). Microsatellites are generally assumed to have evolved neutrally so the frequency and distribution should reflect mutation and drift (Ellegren 2004; but see Buschiazzo & Gemmell 2006; Selkoe & Toonen 2006). Allelic information from microsatellite loci has provided evaluation of relatedness within and between social groups such as ants (Parker *et al.* 1998; Chapuisat & Crozier 2001; Goropashnaya *et al.* 2001), social crab spiders (Evans & Goodisman 2002), rodent species (Ebensperger *et al.* 2004), eiders (McKinnon *et al.* 2006) and bees (Paxton *et al.* 1996).

In order to analyse the length of the PCR products in the ABI 3100 laser detection system, a fluorescent tag was added using a modified method for fluorescent labelling of PCR fragments developed by Schuelke (2000). A nested PCR reaction mixture contained a 1:4 ratio of an extended locus-specific forward primer with an added universal tag [the sequence UEACT for universal *Eco*RI ACT: (5′ GAC TGC GTA CCC AAT TCA CT 3′)], and a 1:1 ratio of the reverse primer, and a labelled universal primer (G5 series dye) that was complimentary to the extension added to the locus-specific forward primer. The reaction mixture (total of 25 μL) contained 1.5 μL of DNA, 12.06 μL of H_2O, 5.0 μL of 5X PCR reaction Buffer (Promega), 2.0 μL of 10 mM dNTPs, 2.5 μL of MgCl, 0.125 μL GoTAQ® (Promega), 0.3125 μL of modified forward primer and 1.25 μL of reverse and universal primers, which were run under modified cycling conditions described by Schuelke (2000). PCR conditions for each primer are as follows: C106, D126, B225 (94 ºC for 5 min, 30 cycles of: 94 ºC for 30 s, 56 ºC for 45 s, 72 ºC for 45 s and eight cycles of: 94 ºC for 30 s, 53 ºC for 45 s and 72 ºC for 45 s and a 72 ºC extension for 30 min). Primers D110 and D112 followed the above cycling conditions except the

annealing temperatures were changed from 56 °C to 57.3 °C. 1.0 μL of PCR product for each marker was added to a 0.75 μL LIZ standard and formamide mix bringing the total volume to 15 μL, which was then multiplexed on an ABI 3100 Prizm Genetic Analyser in the Molecular Biology Research Facilities lab at the University of Tennessee.

Data analysis

To interpret the fragment analysis from the ABI 3100, microsatellite data peaks were scored by Peakscan, a software package available at the Applied Biosystems website (http://marketing.appliedbiosystems.com/mk/get/PS1_login). Each individual was scored for the number of base pair variations at the five loci examined. Out of the 200 individuals collected, 160 individuals had complete allelic data and were used in this analysis with the goal of evaluating the relatedness of these individuals partitioned into 40 pairs each of solitary individuals and multi-female nest individuals. The software package CERVUS (Kalinowski *et al.* 2007) provided summary statistics for the data set including heterozygosity, number of alleles per locus and tests for deviation from Hardy-Weinberg Equilibrium (HWE).

An AMOVA was performed in Arlequin (Excoffier *et al.* 2005) to address the question of whether social and solitary phenotypes represent demographically separated populations. This program partitions the variance among different hierarchical levels: we used partitions between social and solitary phenotypes, among pairs within social and solitary groupings and among individuals within pairs. Significance is assessed by randomization at each hierarchical level (Fitzpatrick 2009). If significant variation occurs between social and solitary groups, then we would infer that the two phenotypes represent distinct evolutionary units. If little difference between the two groups can be found, then we would support the view that the phenotypes represent within-population polymorphism.

Genetic relatedness between individuals in this study is mathematically represented as the probability that individuals share respectively, zero, one or two alleles of each marker tested that are identical by descent (IBD) (Blouin 2003; Kalinowski *et al.* 2006). Several relatedness estimators are used to perform statistical analysis for the IBD probabilities. These use either linear regression (Queller & Goodnight 1989; Lynch & Ritland 1999; Wang 2002) or maximum-likelihood (Kalinowski *et al.* 2006) based methods. We initially applied the two most common regression methods (Queller & Goodnight 1989; Lynch & Ritland 1999) along with the maximum-likelihood based estimator (Kalinowski *et al.* 2006). All three were significantly correlated (Spearman coefficients between 0.61–0.79 for the social data set and 0.82–0.92 for the solitary data set; all P values < 0.0001). We present only the likelihood results. The likelihood estimator has the desirable property of being bounded at 0 and 1 (facilitating its interpretation as a probability). In addition, it is possible to include an assumption that null alleles are present in some or all loci when using the program ML-RELATE (Kalinowski *et al.* 2006) Due to the high likelihood of null alleles in microsatellites, the data was analysed both with and without null alleles, however no significant differences were detected in the output so to be conservative null alleles were assumed present.

We applied a simple randomization test using R (http://www.r-project.org) to determine whether pairs of females collected from the same communal nest were more closely related than random pairs from the population. A null distribution was simulated using both the social and solitary relatedness estimates by randomly forming 40 pairs (with replacement) and calculating the average pairwise relatedness. This procedure was repeated 10 000 times. We then compared the average relatedness of our observed pairs to this distribution of 10 000 averages under the null hypothesis of no population structure, simulated by random pairings of individuals, with replacement drawn from the population. The fraction of simulation replicates possessing greater than the average relatedness of the observed set of pairs then represents a one-tailed P value for the data given the null hypothesis (Good 1994). We applied a second randomization to test whether social pairs tend to be more closely related than pairs of solitary females at similar spatial distance (see sampling above), we simulated the null hypothesis of no relationship between relatedness and social phenotype, while controlling for spatial distance. Here we kept pairs of females together, but randomly re-labelled them as social or solitary. For each of 10 000 replicates, we calculated the difference between the means, the difference between the medians and the test statistic (the maximum rank sum) for the Wilcoxon paired-sample test and compared the simulated distributions of these statistics to their observed values. Conventional t-tests or Wilcoxon tests were not appropriate because the distributions of relatedness were not symmetrical (Zar 1984).

Results

Genetic structure

The AMOVA showed the highest percentage of variation was explained among individuals within pairs (89.2%) with the lowest amount of variation explained between social and solitary groups (0.102%; Table 2). Thus,

there was no detectable population genetic differentiation between social and solitary phenotypes. However, there was significant variation among pairs (10.72%; Table 2), i.e. social individuals sharing a nest or solitary individuals in close proximity were more similar than expected by chance.

Characteristics of the five polymorphic loci used in the analysis of *A. studiosus* kinship patterns are detailed in Table 1. The di-nucleotide repeat markers offered between three and 22 alleles with an average of 12.4 alleles and a mean expected heterozygosity of 0.7195 (Table 1). Deviations from Hardy–Weinberg were evident, with four out of five showing heterozygote deficits overall (Table 1). Heterozygote deficit could be attributed to the presence of (i) null alleles; (ii) non-random mating; or (iii) population subdivision (Wahlund effect). Non-random mating (inbreeding) might be an expected outcome of kin selection and population subdivision is consistent with the AMOVA results. Nevertheless, to be conservative, we specified that these loci be analysed with null alleles assumed present in the maximum likelihood program ML-RELATE (Kalinowski *et al.* 2006).

Relatedness

The average pairwise relatedness for individuals in communal/social nests ranged from zero (unrelated) to 0.85 and had an average of 0.25 (n = 40 pairs). Solitary nests had pairwise estimate relatedness values ranging from zero (unrelated) to 0.71, with an average of 0.18 (n = 40 pairs). The social and solitary mean and median values were significantly different from random (Monte Carlo, 10 000 randomizations), indicating that spiders in the same nests (or in close proximity) are more closely related than random pairs of individuals in this population (Fig. 1).

Table 2 AMOVA for social and solitary pair relatedness coefficients, F statistics and P values from Arlequin

Source of variation	d.f.	% of variation	Fixation index	P value
Between social and solitary phenotypes	1	0.102	F_{CT} = 0.001	0.335
Among pairs within phenotypes	78	10.722	F_{SC} = 0.107	0.018
Within pairs	224	89.175	F_{ST} = 0.108	0.017

The second randomization tested for significant differences between social pairs and solitary pairs of individuals. The proportion of randomizations with median difference greater than or equal to observed median difference (observed = 0.17) was $P < 0.0434$ and for the Wilcoxon paired test statistic (observed = 397.5) $P = 0.0326$. Therefore, social pairs are significantly more related than the solitary pairs in this population, although the difference is subtle (Fig. 2).

For social pairs, the mean relatedness of 0.247 (bootstrap 95% CI: 0.171–0.321) and median of 0.226 (0.110–0.320) are consistent with a half-sibling level of relatedness. Neither confidence interval overlaps 0.5, the expected relatedness of full siblings. For solitary pairs, the mean of 0.182 (0.110–0.253) and median of 0.08 (0.000–0.170) illustrate a right-skewed distribution where most pairs were unrelated.

Discussion

Typically, cooperative social spiders are monomorphic for the social phenotype and exhibit high levels of

Table 1 Characteristics of the five polymorphic loci scored in *A. studiosus* with the primer sequence shown in addition to the most popular allele for the loci in the population (n = 200). Tm (°C) refers to optimizing annealing temperatures. n = number of individuals scored, H_{obs} = observed heterozygote frequency, H_{exp} = expected heterozygote frequency assuming Hardy–Weinberg equilibrium (HWE), and P refers to significance of deviation from HWE following Bonferroni Correction

Locus	Length of fragment (bp)	Number of alleles	Primer sequence (5′_3′)	Tm (°C)	N_{ind}	Hardy-Weinberg H_{obs}	Hardy-Weinberg H_{exp}	Hardy-Weinberg Deviation P-value
C106	137	8	AAGCAAAATGCCTCCTTT GCTCAGAAGACGAGTGATTC	56	137	0.533	0.646	0.045
D126	148	3	CATTGTGCCAAAAGTGTG GCAGTGTTGCTTGTCTGTT	56	148	0.635	0.452	<.0001
B225	146	10	GGCTTCAATGTAATCCAAGTG GCACGCCACTGATATAAATG	56	146	0.315	0.782	<.0001
D110	114	22	GGAGAAATTCTGTCAAATCTGG GGCGATGTTACCTTTATTAACG	57.3	114	0.395	0.844	<.0001
D112	120	19	ATTCCGACTGTCGTATCCTT GCATTTTAGATTCACAGACACC	57.3	120	0.375	0.873	<.0001

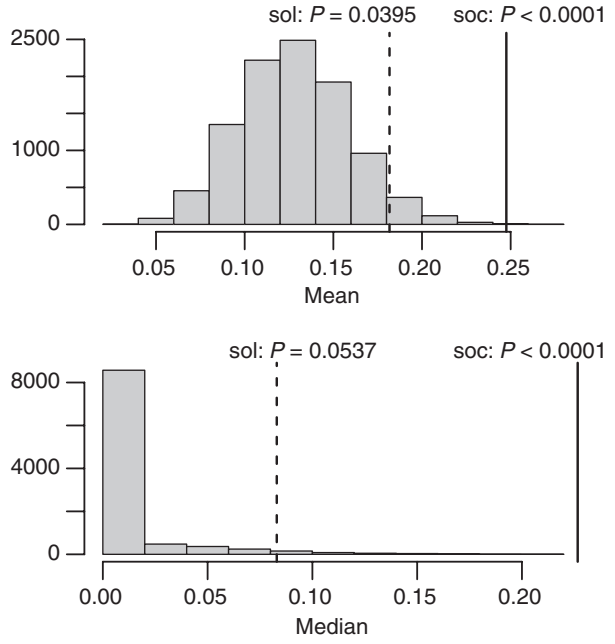

Fig. 1 Monte Carlo simulation (10 000 randomizations) for the median and mean of social and solitary relatedness values. Solitary (sol) is shown with a dashed line and social (soc) with a full line. *P* values are the fractions of the Monte-Carlo null distributions that are greater than or equal to the observed values.

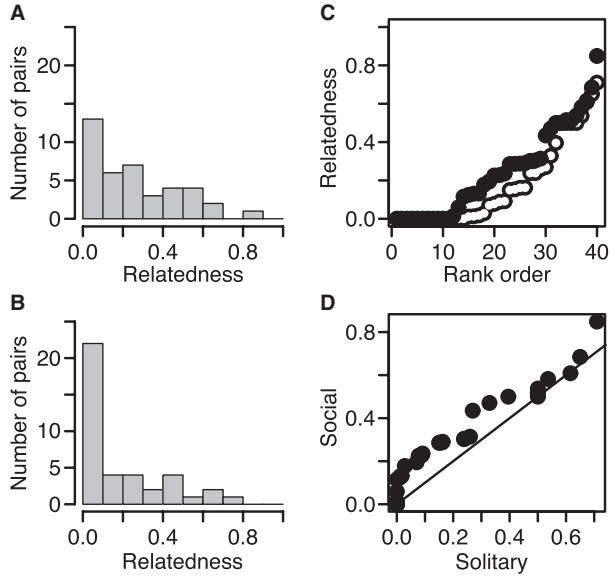

Fig. 2 (A, B) Frequency distributions of pairwise relatedness estimates for social and solitary *A. studiosus*. The order statistics (C) and quantile–quantile plot (D) illustrate that the distribution of relatedness between social individuals is shifted relative to the distribution for pairs of solitary individuals.

inbreeding. Measures of within colony degrees of relatedness are on the order of full sibs or higher (Buskirk 1981; Roeloffs & Riechert 1988; Riechert & Roeloffs 1993; Aviles 1997; Lubin & Bilde 2007). In contrast, our AMOVA results indicate that *A. studiosus* populations are polymorphic for social and solitary phenotypes and our estimates of within-colony relatedness of social *A. studiosus* were closer to the half-sib level on average. For these reasons, *A. studiosus* is an exemplary system in which to examine the forces shaping the evolution and maintenance of sociality.

The evolution of cooperative group behaviour can be explained by mutual benefit and/or increased inclusive fitness of cooperative genotypes (i.e. kin selection). Mutual benefits in *A. studiosus* include increased silk production with more females producing energetically expensive silk and shared brood care if a mother dies before her brood is old enough to care for themselves (Riechert & Jones 2008). Our results on the relatedness of *A. studiosus* are consistent with a role for kin selection in that cooperative females are more closely related than expected by chance and significantly more related than solitary female pairs with similar inter-individual distances. These results suggest that cooperative interactions in *A. studiosus* are likely to benefit kin over and the above the level expected by population structure alone.

Although social pairs were more closely related than solitary pairs, solitary pairs were also more closely related than expected by chance (Fig. 1). This suggests that the intrinsically low dispersal of *A. studiosus* females might pre-dispose these spiders to the evolution of cooperation because interactions are, by default, more likely to involve relatives. Dispersal of *A. studiosus* females is known to average less than 1 m (Riechert & Jones 2008) and this alone might explain spatial genetic structure on the scale of our 100 m transects. However, the relatedness of social pairs is even greater than that of solitary pairs at the same distance, indicating that the benefits of cooperation are likely to benefit kin over and above the level expected based on population viscosity alone.

The range of social behaviour exhibited by *A. studiosus*, with a higher frequency of multi-female nests present at cold-water sites than warm-water sites (Jones *et al.* 2007), suggests that environmental factors maintain behavioural trait variation beyond the indirect benefits conferred by increased relatedness among females within multi-female nests. Direct benefits from cooperation include the sharing of the costs of silk production, which benefits individuals by being able to share the energetic costs of maintaining webs for prey capture and antipredatory benefits (Aviles 1997; Crespi & Choe 1997; Uetz *et al.* 2002; Lubin & Bilde 2007; Yip *et al.* 2008). Additionally, in harsh environments, where dispersal is constrained and the probability of reproduction is low, energetic benefits might

be gained by living in groups (Lubin & Bilde 2007). For example, the brood fostering model proposed by Jones *et al.* (2007) explains variation in the level of sociality based on a modified fitness return strategy (Strassman & Queller 1989) in which females 'hedge their bets' (Soucy 2002) against dying before her brood reaches maturity.

Male vagility and multiple paternity are two factors that might explain the lower relatedness values observed in *A. studiosus* compared to that in the tropical social spider species. Male *A. studiosus* mature earlier than females and leave the natal nest in search of females with a preference for social females exhibited by males from both subsocial and social mothers (Pruitt & Riechert 2009). The strong female biased sex ratios of the tropical social spiders reflect the fact that males stay in the natal nest and mate with sibs (reviewed in Riechert & Roeloffs 1993). Because *A. studiosus* females will accept males as long as 8 days after an initial successful copulation (Sarah I. Duncan, personal observation), this too might lead to mating with multiple males. This decreases the average relatedness of the offspring.

This study was aimed at evaluating whether kin selection might contribute to the evolution and maintenance of cooperative behaviour in a social spider. Our estimates of pairwise relatedness support a role for kin selection in that social pairs tend to be more closely related than solitary pairs. However, solitary pairs (at similar inter-individual distances) are also more closely related than expected in a panmictic population, indicating that low dispersal could facilitate the evolution of cooperation in this group of spiders. In addition, relatedness in *A. studiosus* is considerably lower than that reported for non-polymorphic social spiders in the tropics. This difference might reflect differences in the costs and benefits involved in social behaviour or might indicate that the extreme degree of kinship and inbreeding observed in some tropical colonies is a secondary effect of colonial behaviour in those systems. *Anelosimus studiosus* presents a unique opportunity to examine the evolution of cooperative behaviour in its early stages because sociality exists as a polymorphism within populations and both environmental and genetic factors might contribute to the current distribution of phenotypes.

Acknowledgements

We would like to thank the Riechert, Fitzpatrick and Fordyce Lab members for their support, ideas and helpful discussion. We thank Jubal Duncan and Jonathon Pruitt for aiding in collections of *A. studiosus*. We would also like to thank Stesha Pasachnik for providing advice in the molecular lab and J.R. Jones for his statistical advice. Additionally, we would like to thank Kermit Ritland for his correspondence. This project was funded by NSF grant # 0235311 from the Animal Behavior Program of the National Science Foundation. Additionally, we would like to thank the three anonymous reviewers for their helpful comments and advice.

References

Agnarsson I, Maddison WP, Aviles L (2006) The phylogeny of the social *Anelosimus* spiders (Aranea: Theridiidae) inferred from six molecular loci and morphology. *Molecular Phylogenetics and Evolution*, **43**, 833–851.

Aviles L (1997) The causes and consequences of cooperation and permanent sociality in spiders. In: *The Evolution of Social Insects and Arachnids* (eds Choc JC, Crespi BJ), pp. 476–498. Cambridge University Press, Cabbridge, UK.

Baglione V, Canestrari D, Marcos JM, Ekman J (2003) Kin selection in cooperative alliances of Carrion crows. *Science*, **300**, 1947–1949.

Blouin MS (2003) DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. *Trends in Ecology and Evolution*, **18**, 503–511.

Blouin MS, Parsons M, Lacaille V, Lotz S (1996) Use of microsatellite loci to classify individuals by relatedness. *Molecular Ecology*, **5**, 393–401.

Brach V (1977) *Anelosimus studiosus* (Araneae: Theridiidae) and the evolution of quasisociality in theridiid spiders. *Evolution*, **31**, 154–161.

Burland TM, Bennett NC, Jarvis JUM, Faulkes CG (2002) Eusociality in African mole-rats: new insights from patterns of genetic relatedness in the Damarland mole-rat (*Cryptomys damarensis*). *Proceedings of the Royal Society B: Biological Sciences*, **269**, 1025–1030.

Buschiazzo E, Gemmell NJ (2006) The rise, fall and renaissance of microsatellites in eukaryotic genomes. *Bioessays*, **28**, 1040–1050.

Buskirk RE (1981) Sociality in the arachnida. In: *Social Insects* (ed. Herman HR) pp. 282–376 Academic Press, London.

Chapuisat M, Crozier R (2001) Low relatedness among cooperatively breeding workers of the greenhead and *Rhytidoponera metallica*. *Journal of Evolutionary Biology*, **14**, 564–573.

Crespi BJ, Choe JC (1997) Explanation and evolution of social systems. In: *The Evolution of Social Insects and Arachnids* (eds Choc JC, Crespi BJ), pp. 476–498. Cambridge University Press, Cambridge, UK.

Csilléry K, Johnson T, Beraldi D *et al.* (2006) Performance of marker-based relatedness estimators in natural populations of outbred vertebrates. *Genetics*, **174**, 2091–2101.

Ebensperger LA, Hurtado MJ, Soto-Gamboa M, Lacey EA, Chang AT (2004) Communal nesting and kinship in degus (*Octodon degus*). *Naturwissenchaften*, **91**, 391–395.

Ellegren H (2004) Microsatellites: simple sequences with complex evolution. *Nature Reviews*, **5**, 435–445.

Evans TA, Goodisman MAD (2002) Nestmate relatedness and population genetic structure of the Australian social crab spider *Diaea ergandros* (Araneae: Thomisidae). *Molecular Ecology*, **11**, 2307–2316.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.

Fitzpatrick BM (2009) Power and sample size for nested analysis of molecular variance. *Molecular Ecology*, **18**, 3961–3966.

Foster KR, Wenseleers T, Ratnieks FLW (2006) Kin selection is the key to altruism. *Trends in Ecology and Evolution*, **21**, 57–60.

Furey RE (1998) Two cooperatively social populations of the theridiid spider *Anelosimus studiosus* in a temperate region. *Animal Behavior*, **55**, 727–735.

Futuyma DJ (2009) *Evolution*, 2nd edn. Sinauer Associates, Inc., Sunderland, MA.

Good P (1994) *Permutation Test: A Practical Guide to Resampling Methods for Testing Hypotheses*, 2nd edn. Springer, New York.

Goropashnaya AV, Seppa P, Pamilo P (2001) Social and genetic characteristics of geographically isolated populations in the ant *Formica cinerea*. *Molecular Ecology*, **10**, 2807–2818.

Hamilton WD (1964) The gentical evolution of social behaviour. *Journal of Theoretical Biology*, **7**, 1–16.

Hughes WOH, Oldroyd BP, Beekman M, Ratnieks FLW (2008) Ancestral monogamy shows kin selection is key to the evolution of eusociality. *Science*, **320**, 1213–1216.

Johannesen J, Lubin Y (2001) Evidence for kin-structured group founding and limited juvenile dispersal in the subsocial spider *Stegodyphus lineatus* (Aranea, Erasidae). *The Journal of Arachnology*, **29**, 413–422.

Johannesen J, Baumann T, Seitz A, Veith M (1998) The significance of relatedness and gene flow on population genetic structure in the subsocial spider *Eresus cinnaberinus* (Araneae: Eresidae). *Biological Journal of the Linnaean Society*, **63**, 81–98.

Jones TC, Riechert RE. (2008) Patterns of reproductive success associated with social structure and microclimate in a spider system. *Animal Behaviour*, **76**, 2011–2019.

Jones TC, Riechert SE, Dalrymple S, Parker PG (2007) Fostering model explains variation in level of sociality in a spider system. *Animal Behaviour*, **73**, 195–204.

Kalinowski ST, Wagner AP, Taper ML (2006) ML-RELATE: a computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes*, **6**, 576–579.

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, **16**, 1099–1006.

Kümmerli R, Keller L (2007) Contrasting population genetic structure for workers and queens in the putatively unicolonial ant *Formica exsecta*. *Molecular Ecology*, **16**, 4493–4503.

Lubin Y, Bilde T (2007) The evolution of sociality in spiders. *Advances in the Study Behavior*, **37**, 83–145.

Lynch M, Ritland K (1999) Estimation of pairwise relatedness with molecular markers. *Genetics*, **152**, 1753–1766.

McKinnon LH, Gilchrist G, Scriber KT (2006) Genetic evidence for kin-based female social structure in common eiders (*Somateria mollissima*). *Behavioral Ecology*, **17**, 614–621.

Parker PG, Snow AA, Schug MD, Booten GC, Fuerst PA (1998) What molecules can tell us about populations: choosing and using a molecular marker. *Ecology*, **79**, 361–382.

Paxton RJ, Thoren PA, Tengo J, Estoup A, Pamilo P (1996) Mating structure and nestmate relatedness in a communal bee, *Andrena Jacobi* (Hymenoptera, Andrenidae), using microsatellites. *Molecular Ecology*, **5**, 511–519.

Pruitt JN, Riechert SE (2009) Male mating preference is associated with the risk of pre-copulatory cannibalism in a socially polymorphic spider. *Behavioral Ecology and Sociobiology*, **63**, 1573–1580.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.

Riechert SE, Jones TC (2008) Phenotypic variation in the social behavior of the spider *Anelosimus studiosus* along a latitudinal gradient. *Animal Behaviour*, **75**, 1893–1902.

Riechert SE, Roeloffs R (1993) Inbreeding and its consequences in the social spiders. In: *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives* (ed Thornhill NW), pp. 283–303. University of Chicago Press, Chicago.

Roeloffs R, Riechert SE (1988) Dispersal and population-genetic structure of the cooperative spider, *Agelena consociate*, in the West African Rainforest. *Evolution*, **42**, 173–183.

Ross KG (2001) Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. *Molecular Ecology*, **10**, 265–284.

Safi K, Kerth G (2007) Comparative analyses suggest that information transfer promoted sociality in male bats in the temperate zone. *American Naturalist*, **170**, 465–472.

Scantlebury M, Bennett NC, Speakman JR, Pillay N, Schradin C (2006) Huddling in groups leads to daily energy savings in free living African Four-Striped Grass Mice, *Rhabdomys pumilio*. *Functional Ecology*, **20**, 166–173.

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology*, **18**, 233–234.

Schwarz MP, Richards MH, Danforth BN (2007) Changing paradigms in insect social evolution: insights from Halictine and Allodapine bees. *Annual Review of Entomology*, **52**, 127–150.

Selkoe KA, Toonen RJ (2006) Microsatellites for ecologist: a practical guide to using and evaluating microsatellite markers. *Ecology Letters*, **9**, 615–629.

Shavit A, Millstein RL (2008) Group selection is dead! Long live group selection. *BioScience*, **58**, 574–575.

Smith DR, Hagen RH (1996) Population structure and interdemic selection in the cooperative spider *Anelosimus eximius*. *Journal of Evolutionary Biology*, **9**, 589–608.

Smith AR, Wcislo WT, O'Donnell S (2007) Survival and productivity benefits to social nesting in the sweat bee *Megalopta ganalis* (Hymenoptera: Halictidae). *Behavioral Ecology and Sociobiology*, **61**, 1111–1120.

Smith D, Van Rijn S, Henschel J, Bilde T, Lubin Y (2009) Amplified fragment length polymorphism fingerprints support limited gene flow among social spider populations. *Biological Journal of the Linnean Society*, **97**, 235–246.

Soro A, Ayasse M, Zobel MU, Paxton RJ (2009) Complex sociogenetic organization and the origin of unrelated workers in a eusocial sweat bee, *Lasioglossum malachurum*. *Insectes Sociaux*, **56**, 55–63.

Soucy SL. (2002) Nesting biology and socially polymorphic behavior of the sweat bee *Halictus rubicundus* (Hymenoptera: Halictidae). *Annals of the Entomological Society of America*, **95**, 57–65.

Strassman JE, Queller DC (1989) Ecological determinants of social evolution. In: *The Genetics of Social Evolution* (eds Breed MD, Page RE Jr), pp. 81–101. Westview Press, Boulder, Co

Strassman JE, Hughes CR, Turillazzi S, Solís CR, Queller DC (1994) Genetic relatedness and incipient eusociality in stenogastrine wasps. *Animal Behaviour*, **48**, 813–821.

Uetz GW, Boyle J, Hieber CS, Wilcox RS (2002) Anti-predator benefits of group living in colonial web building spiders- the ''Early Warning'' effect. *Animal Behaviour*, **63**, 445–452.

Vucetich JA, Peterson RO, Walte TA (2004) Raven scavenging favours group foraging in wolves. *Animal Behaviour*, **67**, 1117–1126.

Wang J (2002) An estimator for pairwise relatedness using molecular markers. *Genetics*, **160**, 1203–1215.

Whitehouse MEA, Lubin Y (2005) The functions of societies and the evolution of group living: a spider societies as a test case. *Biological Review*, **80**, 347–361.

Wilson EO, Hölldobler B (2005) Eusociality: origin and consequences. *Proceedings of the National Academy of Science*, **102**, 13367–13371.

Wilson SL, Wilson EO (2007) Rethinking the theoretical foundation of sociobiology. *The Quarterly Review of Biology*, **82**, 327–348.

Yip EC, Powers KS, Aviles L (2008) Cooperative capture of large prey solves scaling challenge faced by spider societies. *Proceedings of the National Academy of Sciences*, **105**, 11818–11822.

Zane L, Bargelloni L, Patarnello T (2002) Strategies for microsatellite isolation: a review. *Molecular Ecology*, **11**, 1–16.

Zar JH (1984) *Biostatistical Analysis*, 2nd edn. Prentice Hall, New Jersey.

S. Duncan studied genetic structure in socially polymorphic spiders for her MS thesis and is interested in behavior and conservation genetics. S. Riechert's work is at the interface between behavior, ecology and evolutionary biology and involves the extent to which populations are at adaptive equilibria with respect to their physical and biotic environments. B. Fitzpatrick's major interests are in genetics and biogeography of speciation, the importance of local adaptation for both evolution and conservation management, and invasion biology. J. Fordyce's interests are the underlying processes responsible for the evolution of behavioral, ecological, physiological and morphological discontinuities in nature, and how these processes might ultimately affect reproductive isolation.
