



Phenotypic variation in the social behaviour of the spider *Anelosimus studiosus* along a latitudinal gradient

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We investigated the behavioural mechanism underlying the recently discovered latitudinal variation in the social structure of the spider *Anelosimus studiosus* through population censuses, behavioural interaction trials, reciprocal nest transplants to different temperature environments and breeding experiments. Nest censuses completed at replicated sites at 2° changes in latitude between south Florida (26°) and east Tennessee (36°) indicated that the dominant social structure is a solitary female nest. Multifemale nests consisting of cooperative females and their young first appeared in the populations at 30° latitude and increased in frequency of representation with further increases in latitude as did number of females within multifemale nests. Interaction trials showed that communication and physical contact underlie the two social structures. Females from solitary female nests and F1 offspring reared in the laboratory demanded space (asocial phenotype), whereas individuals of multifemale nest origin (from same and foreign nests) were attracted to one another (social phenotype). Field experiments further showed that (1) individuals that dispersed from multifemale nests dispersed shorter distances and (2) nest coalescence was observed only at the higher latitudes, coincident with the presence of multifemale nests. Habitat transplants suggested that the behaviour underlying social structure is not plastic in this system, and breeding experiments eliminated a cryptic species alternative to the presence of different social structure phenotypes. Although both asocial and social phenotypes were present at all latitudes, the frequency of the social phenotype was rare at lower latitudes.

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The social structure or organization expressed within local populations of a species is important because it reflects adaptive responses to environmental constraints just as any morphological or physiological trait value would. Because social organization is subject to selection, it often varies among species in a lineage or between closely related lineages, and studies of the origins of sociality usually involve species lineage comparisons. Fewer studies emphasize population variation within a species, and the study of within-population variation is unusual. However, social polymorphism has been described in many species

of halictid bees with some showing phenotypic variation within populations (see reviews in: Danforth & Eickwort 1997; Wcislo 1997). Halictids have presented a unique system with which to explore the ecological correlates and other factors underlying social structure.

Two recent studies have identified a spider species that, like some halictid species, shows evidence of within- and between-population social polymorphism (Furey 1998; Jones et al. 2007). From work completed in south Florida, *Anelosimus studiosus* had been categorized as a subsocial species in which the solitary female provides extended care of its offspring (Brach 1977). Furey (1998) first reported evidence of multifemale colonies in east Tennessee, and Jones et al. (2007) presented a brood-fostering model that predicts that colder environmental temperatures favour cooperative living at higher latitudes for *A. studiosus*. This spider provides an excellent opportunity to examine the mechanistic pathway from a solitary, competitive social structure

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to a communal cooperative one in an arthropod species that lacks the complexities of an haplodiploid inheritance pattern. *Anelosimus studiosus* populations are often quite large and extensive, thus allowing quantification of the frequencies of social behaviour phenotypes across the species range. The goal of this study was to document the latitudinal patterns of social structure in this species and to gain insight into the extent to which the expression of social behaviour reflects phenotypic plasticity.

METHODS

Target Species

Anelosimus studiosus is an annual tropical/temperate species distributed from Argentina in South America to New England in North America (Levi 1956; Agnarsson 2006). Unlike most spiders, the mother's life span overlaps the emergence of her offspring, during which she guards and regurgitates food to them over an extended period of dependence. The young are driven off as they become mature (males at six instars, females at seven instars). The female may produce up to three broods during her annual life span. At the mother's death, the nest often is taken over by a dominant female offspring who forces the dispersal of its sibs.

The phenology of this species varies with latitude. At the southern latitudes in the U.S.A. (i.e. 26–28°), nests at all stages of colony development can be found at any given time as the warm temperatures support continuous spider activity. With increasing latitude, colony growth becomes increasingly more synchronous. At the highest latitude included in this study (36°), females mature in late May and produce clutches in June and early July. All adult females are deceased by the end of August and

dispersal of late-instar offspring occurs from August into the autumn.

Study Areas

Two replicate sites were identified at every 2° change in latitude from the Everglades in south Florida (26°) to east Tennessee (36°) as shown in Fig. 1. In choosing sites, we searched for large populations of *A. studiosus* positioned along a linear transect that could easily be accessed. As this spider apparently has an affinity for water, all sites were located either along the margins of water bodies where they could be visited by boat (higher latitudes), or along the shrub line associated with a road adjacent to a waterway (Everglades). We attempted to locate replicate sites that were approximately 50–75 km apart. All of the lake transects listed in Fig. 1 with the exception of Lake Louisa in central Florida were located on reservoirs, created by impounding waterways.

Transect Censuses

At each of these sites we completed censuses of *A. studiosus* nests and associated habitat at 1 m intervals over a continuous transect of 500 m. The latitudinal and longitudinal coordinates presented in Fig. 1 identify the starting points of each of the transects. The coordinates were obtained using a Garmin Etrex GPS unit. A permanent rebar stake marked these starting points, thus allowing for repeated sampling. The metre interval sample followed the outside of a vegetation boundary (shrub/tree line) along a waterway or road. We counted all nests in this sample that were located within 2 m above the substrate and that were at a depth into the vegetation of

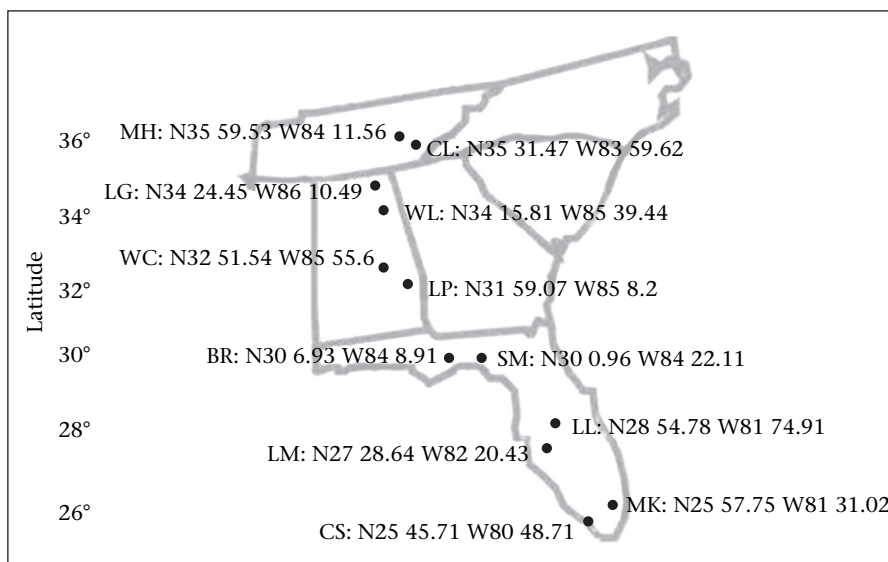


Figure 1. Location of *Anelosimus studiosus* study areas along a latitudinal transect in southeastern U.S.A. Site abbreviation: latitude and longitude. From lower to higher latitude: Florida: CS = Collier-Seminole State Park; MK = Miccosukee Indian Reservation; LM = Lake Manatee State Recreation Area; LL = Lake Louisa State Park; SM = St Marks National Wildlife Refuge; BR = Bottoms Road tract of St Marks Wildlife Refuge; Alabama: LP = Lake Point State Park; WC = Wind Creek State Park; WL = Weiss Lake; LG = Lake Guntersville State Park; Tennessee: MH = Melton Hill Lake; CL = Chilhowee Lake.

less than 1 m. These limits reflect constraints on our observational abilities, particularly when sampling by boat. The habitat parameters measured along these transects are listed elsewhere (T. C. Jones, S. E. Riechert & A. R. Mains, unpublished data).

Nest Composition

We collected 25 *A. studiosus* nests coincident with the production of clutches by adult females at particular latitudes. We started the censuses in March 2004 at 26° and successively sampled higher latitudes through late spring into June. At the time of collection, we used a random draw method to determine the respective metre intervals from which to take nests. If we did not find a nest in a designated interval, we drew a new interval for that nest. If an interval had more than one nest present, we chose the most centrally placed nest within that interval. We estimated nest size in situ by measuring its maximum length, width and height in centimetres where nest volume (cm^3) = $W \times L \times H$. We then clipped each nest from the branch of the tree, shrub, herb or palm that it was in and bagged it for return to our field laboratory for dissection. We recorded the number and age of *A. studiosus* individuals present in each of these collected nests. The spiders from these nests were then used as test subjects in the experiments described below with additional nest collections made throughout the study as sample size needs dictated. The additional nests were collected from surrounding areas rather than from transect intervals.

Social Structure: Spider Interaction Test

The nest dissections provided information on frequencies of solitary versus social *A. studiosus* nests at each site and latitude. We completed spider interaction trials to quantify the frequencies of potential behavioural phenotypes that might underlie the two social structures: solitary/territorial (demanding space) versus communal/cooperative (attracted to conspecifics). These trials were completed in clear plastic boxes, measuring 10 cm on a side and 3 cm in height. *Anelosimus studiosus* requires a structure in which to build its scattered-line web and thus is attracted to the corners of a box (Fig. 2). We assumed that, if *A. studiosus* is indifferent to conspecifics, then two individuals released into the test arena would by chance settle in the same corner or opposite corner from one another with probabilities of 0.25. On the other hand, the chance of settling in adjacent corners would be 0.50 because adjacent corners were offered with a frequency equal to two times that of same and opposite corners.

All test subjects were fed 24 h before their use in these trials. Following the release of a pair of individuals matched for age and size in the centre of the arena, we replaced the cover on the box and set it aside for 24 h. (Note that no statistical differences in trial outcome were detected between classes of females used in these trials: penultimate, unmated mature, gravid and with egg case: $\chi^2_3 = 0.67$.) At the end of this 24-h period, we recorded all mortality suffered and the relative positions of the

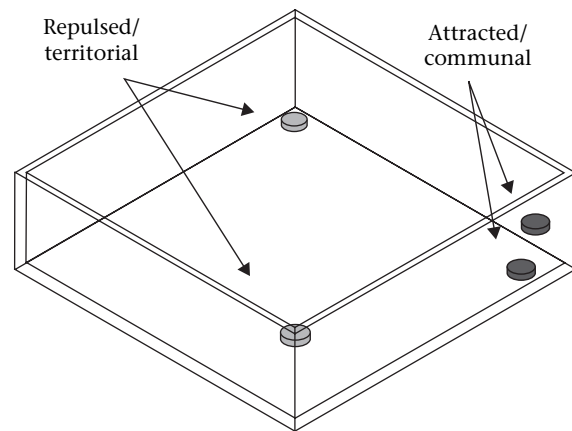


Figure 2. Diagram of interaction trial arena with potential outcomes: same corner = 'attracted' phenotype characteristic of communal social structure; opposite corners = 'repulsed' phenotype characteristic of territorial social structure. Two spiders paired for age and size were released in the centre of the arena at the start of a trial.

two living individuals as being on same side or corner of the arena (attracted to one another: 'social' phenotype), adjacent sides or corners (no assigned behavioural type), or opposite sides or corners (demanding space: 'asocial' phenotype) (Fig. 2). We also measured the horizontal and vertical distances between individuals (in mm) and converted these to linear distances using the Pythagorean Theorem for right triangles. This permitted us to assign trial outcomes to the occasional individuals that failed to settle in corners. For the first 10 trials of a given sample, we additionally completed a 10 min watch of the individuals at the time of release. We recorded all actions by each individual over this watch period as well as the position of the individuals relative to one another at the end.

We applied this interaction test delineating social phenotype to several classes of individuals. First, we completed three sets of trials, all involving penultimate to adult, field-collected females: (1) between females from different solitary nests within the same population ($N = 50$ trials for each population at each latitude), (2) between females from the same multifemale nest ($N = 25$) and (3) between females collected from different multifemale nests ($N = 25$) (five different local populations at 36° latitude). We also completed the interaction test on laboratory-reared F1 generation penultimate to adult females originating from the six latitudes. F1 test subjects came from clutches that were produced by brooding females collected from the field. The juveniles were reared with their mothers under unlimited food in the laboratory. As each individual reached the fifth instar, it was removed from the home nest and reared in isolation until its use in an interaction trial.

Breeding Test for Cryptic Sister Species

Examination of the genitalia of spiders from each site at the six latitudes included in this study failed to provide evidence of the presence of two species. We completed a cross-latitude breeding experiment to test the hypothesis that individuals maintaining solitary nests and those

occupying multifemale nests represent cryptic sister species. Matings designated as controls were completed between individuals collected at each latitude along the gradient from 26° in south Florida to 36° in east Tennessee. Reciprocal far cross (10° latitude difference: low (26–28°) × high (34–36°)) and near cross (4° latitude difference: low × mid (26° × 30°) and high × mid (36° × 32°)) mating opportunities were also completed. For the far crosses, we used individuals collected from multifemale nests as the source of the high-latitude parents: all low-latitude parents originated from single-female nests. We recorded all mating opportunities as to success or failure and, for successful matings, collected data on the viability of clutches produced, offspring numbers and proportion of offspring surviving to maturation.

Field Test for Phenotypic Plasticity in Social Structure

A four by four, reciprocal transplant experiment was conducted at 36° latitude, involving two cold-water sites (22°C = average summer temperature) and two warm-water sites (27°C). The cold-water sites were located below the dams impounding latitudinal transect areas MH and CL (Fig. 1) and are identified here as MH_c and CL_c, respectively. The two warm-water sites were along Ish Creek (ISH) (35°47.4'N, 84°06.81'W, elevation 257 m) and at I.C. King Park (IK) (35°42.58'N, 84°02.34'W, elevation 247 m). At each site, we collected colonies consisting of fourth- to fifth-instar *A. studiosus* in early autumn (second week in September) when most mothers have died. From preliminary censusing, we identified sections of ISH and IK sites for use in this experiment that had only solitary nests. It was not possible to find locations at the cold-water sites that had only multifemale colonies. At these latter sites we marked multifemale colonies earlier in the year (June–July) to ensure that we would be collecting young from only multifemale nests when we revisited the sites in the autumn.

We brought the colonies back to the laboratory for dissection and used the juveniles obtained from these nests to reconstitute new colonies consisting of 15 individuals of like site and nest type (solitary female nest origin versus multifemale nest origin). These clusters of individuals were forced to build new webs on sprigs of Virginia pine, *Pinus virginiana* (a known preferred habitat) in plastic containers. After 3 days when most colonies had formed nests on the sprigs, we wired them into preferred vegetation (Virginia pine and Chinese privet, *Ligustrum sinense*) at the CL, MH, ISH and IK field sites, a method shown to be successful in this species (Jones & Parker 2002). Each of the four field sites received 20 reconstituted nests from each of the four source sites, for a total of 80 transplanted nests at each site. Thus, at a given site, one-fourth of the introduced individuals originated from nests collected at that site, and one-half of the introduced individuals originated from nests that showed the same general temperature environment and should the same social structure.

We tagged each reconstituted colony on release to identify its source population and assigned it a unique number, reflecting its transplant site. Care was taken to ensure that no other colonies (natural or reconstituted) were within 5 m of the transplanted colony. This distance is based on the results of Powers & Aviles' (2003) study of dispersal of juveniles from the nests of *A. jucundus* (a western U.S.A. *Anelosimus* species that also shows extended maternal care). In their study, initial dispersal distances did not exceed 5 m. From this information, we assumed that any new colonies of *A. studiosus* found within 2 m of a transplanted colony could be labelled as a dispersal event.

Two weeks after transplanting the nests, we checked them for survivorship and movement that might have resulted from the disruption of the transplant process. We checked the transplant locations again the following June after the transplanted spiders had matured and would have dispersed from the natal nest had they been inclined to do so. We measured the distance of all new nests located within 5 m of each tag demarcating the site of a particular transplant. All of these nests were then collected and dissected in the laboratory where we recorded the numbers and ages of spiders found in each nest relative to its source population and transplant site.

Latitudinal Variation in Nest Integrity

We located 30 focal nests containing a brooding female at each 2° change in latitude between 26° and 36°. For inclusion in the census, each nest had to be accessible for visual inspection. At the initial survey, we recorded nearest-neighbour nests in all four compass directions from the focal nest. We also recorded the distance (in cm) to the nearest nest below and above the focal nest. This permitted us to test for potential coalescence between neighbouring nests. We checked for nest movement coalescence and extinction each month over a 4-month period.

RESULTS

Nest Counts and Characterization

Nest densities were generally high, especially given that the estimates were metre interval counts recorded over a 500 m transect (mean number of nests/m: range 0.62–1.65; Table 1). An ANOVA, nesting site within latitude, produced a highly significant whole model result indicating that significant site and latitudinal variation existed in nest densities. A least-square means differences test (Student's *t*) was applied to the nest counts by latitude. The results of this test indicated that the lowest latitude sites (26°) had significantly (at an alpha of 0.05) higher densities of *A. studiosus* nests than did sites censused at mid (30–32°) and high (34–36°) latitudes (Table 1). Nest densities at 28° were intermediate and were statistically non-significantly different from either grouping.

Multifemale nests were first encountered in the census results at 30° and increased in frequency with subsequent

Table 1. *Anelosimus studiosus* nest statistics for site and latitude: data from 500 m transects except for number of females present in multifemale nests where sample sizes were augmented from collections made outside of transect boundaries

Latitude and site	No. nests/m (mean±SE)	Max no. nests/m	Test for nest*	Proportion of multifemale nests	No. females/ multifemale nest (mean±SE)	Test for female*	Max. no. females
26°	1.62±0.08	24	A	0.000			1
Collier-Sem.	1.65±0.11	24		0.000			
Miccosukee	1.60±0.12	16		0.000			
28°	0.62±0.04	11	A B	0.000			1
Manatee	0.33±0.03	6		0.000			
Louisa	0.91±0.07	11		0.000			
30°	0.70±0.06	11	B	0.006	2.38±1.07	A	4
St Marks	0.59±0.08	15		0.008			
Bottoms	0.82±0.08	12		0.004			
32°	0.82±0.06	23	B	0.041	2.47±1.27	A	5
Lake Point	0.61±0.07	20		0.010			
Wind Creek	1.03±0.09	23		0.060			
34°	0.62±0.06	23	B	0.067	3.90±0.91	A B	15
Weiss	0.51±0.09	23		0.028			
Guntersville	0.73±0.09	22		0.091			
36°	0.66±0.06	21	B	0.145	5.89±0.81	B	40
Melton Hill	0.64±0.08	21		0.122			
Chilhowee	0.69±0.07	18		0.166			

*Levels not connected by the same letter were significantly different at $\alpha = 0.05$.

increases in latitude (Table 1). Solitary female nests, nevertheless, were the prominent nest type at all sites. The number of adult females present per nest in the multifemale nests differed between sites within latitudes ($F_{1,92} = 4.40$, $P < 0.0002$) and among latitudes ($F_{5,88} = 3.08$, $P < 0.04$). Least-square means differences test results identified the mid-latitude sites (30° and 32°) as having significantly fewer females/nest (at an alpha of 0.05) from the sites at 36° latitude with respect to the number of females constituting multifemale nests: the 34° latitude sites were intermediate, with numbers of females/nest statistically nonsignificantly different from either the mid-latitude or the highest-latitude sites (Table 1).

Finally, nest volume increased significantly with latitude (Fig. 3a). Nest volume at the high-latitude sites reflected only the number of adult females present (regression relationship: nest volume (cm^3) = 3110.1 (number of females) - 3554.6; $R^2 = 0.89$; Fig. 3b). At low-latitude sites, a regression applied to nest volume identified the numbers of early and late-instar juveniles as significant contributors to nest volume along with the presence or absence of the single mother at an alpha of 0.05 (regression relationship: nest volume (cm^3) = 14.8 (number of early instar juveniles) + 72.0 (number of late-instar juveniles) + 264.7 mother + 11.3; $R^2 = 0.61$).

Social Phenotypes: Interaction Trials

For all sites, the positions of paired adult females in the square box arenas at the end of a 24 h establishment period differed from the expected 0.25 same, 0.25 opposite and 0.5 adjacent corner frequencies at an alpha of at least 0.05 following Bonferroni correction. These field-collected

females tended to be either in the same corner, reflecting attraction as would be the case in a communal social structure, or in opposite corners, reflecting a demand for space as in a territorial social structure (Fig. 4a). There were also highly significant latitudinal differences in relative female positioning in the trials ($\chi^2_{10} = 137.3$, $P < 0.0001$): the shift in positioning was from occupying opposite corners (demanding space) at lower latitudes to occupying the same corner (attraction) at higher latitudes (Fig. 4a). Observations of individual behaviour during the first 10 min following release supported the assumption that individual positioning within the arena resulted from actual communication/engagement between the two individuals. Some form of interaction between individuals during the observation period occurred in 87.9% of the trials. Furthermore, females that failed to interact with one another within the 10 min observation period accounted for 86% of the changes in position from the initial recording at 10 min to that at 24 h.

The results of interaction trials completed on F1 generation laboratory-reared individuals showed a pattern similar to that observed for field-collected females (Fig. 4b). Female positioning differed significantly from random expectations in all groupings (low latitude (26–28°): $N = 55$, $\chi^2_2 = 18.0$, $P < 0.0001$; mid latitude (30–32°): $N = 130$, $\chi^2_2 = 10.4$, $P < 0.01$; high latitude (34–36°): $N = 123$, $\chi^2_2 = 21.6$, $P < 0.0001$). An $R \times C$ chi-square test comparing latitudes indicated that the frequency distribution of female positioning differed significantly between latitudes ($\chi^2_4 = 43.24$, $P < 0.0001$). The preponderance of opposite positioning among individuals from low latitudes and of higher than expected same corner positioning among F1 females from high latitudes were the major contributing factors to this test result.

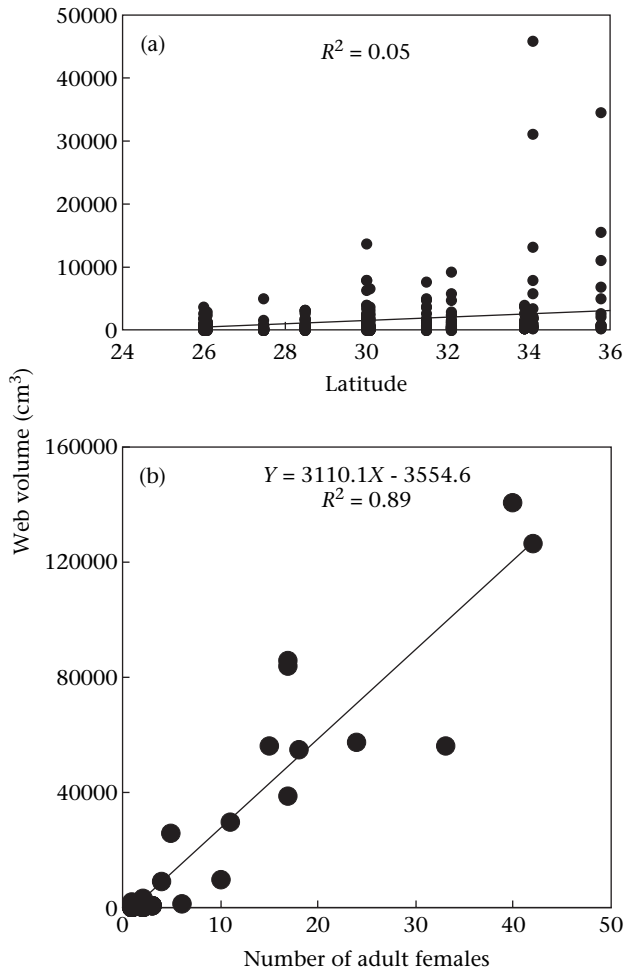


Figure 3. Nest volumes recorded at study sites along a latitudinal transect from south Florida (26°) to east Tennessee (36°): (a) nest volume by latitude (transect censuses only); (b) nest volume at high-latitude sites relative to the number of adult females present (transect and experimental nest measurements made at 36° latitude sites MH and CL).

We also examined nest context using field-collected adult females from 36° latitude sites showing a mixture of solitary female and multifemale nests (Fig. 4c). Interaction results for paired females of solitary nest origin differed from those for adult female nestmates collected from multifemale nests ($\chi^2_4 = 283.2$, $P < 0.0001$) and from those for adult females collected from different multifemale nests ($\chi^2_4 = 247.53$, $P < 0.0001$). The two multifemale trial types did not differ significantly from one another ($\chi^2_4 = 2.67$, $P < 0.27$).

Breeding Test for Cryptic Sister Species

Mating success varied from 51 to 83% across the various classes, with the highest success rates observed in the far population crosses (parents from latitudes separated by 10°) (Table 2). Among the parameters measured, only mating success differed significantly between classes ($\chi^2_7 = 21.2$, $P < 0.05$ following Bonferroni correction):

tests for breeding class differences in the frequency of production of a viable clutch, in offspring number and in the proportion of offspring surviving to maturation produced nonsignificant results (Table 2). There were also no significant directional effects identified for reciprocal crosses involving mating opportunities between individuals from near (4° latitude difference) and far (10° latitude difference) latitudes.

Field Test for Phenotypic Plasticity in Social Structure

At the 2-weeks post-transplant check, we found that most (mean \pm SE proportion: 0.98 ± 0.03) of the 20 reconstituted nests from each source population transplanted to each of the four sites had established themselves, as evidenced by the expansion of the nests onto the surrounding vegetation. An ANOVA performed on proportion survived produced a nonsignificant whole model result ($F_{7,315} = 1.3$, $P < 0.35$) as well as nonsignificant source population ($F_{3,317} = 1.1$, $P < 0.45$) and transplant site ($F_{3,317} = 1.3$, $P < 0.35$) effects. However, five of the 320 transplanted nests were lost at this time because the permanent tags were not relocated.

Ultimately, an average of 8.4 ± 0.9 of the reconstituted nests survived the winter to produce successful colonies the following summer. No significant source population or transplant site differences were observed in nest site survivorship (ANOVA: $F_{7,315} = 1.8$, $P < 0.25$; source effect: $F_{3,317} = 0.68$, $P < 0.6$; transplant effect: $F_{3,317} = 1.8$, $P < 0.1$). An ANOVA performed on the dispersal distances of late-instar juveniles from the home nest (distances of new nests from tagged nests) did produce a highly significant whole model result ($F_{7,125} = 9.06$, $P < 0.0001$) and a significant source population effect ($F_{3,129} = 8.7$, $P < 0.0001$). Analysis of variance contrasts completed on source population revealed that dispersers from multifemale nests from the cold-water source sites dispersed significantly shorter distances than did dispersers from solitary female nests from warm-water source sites ($F_{3,129} = 23.8$, $P < 0.0001$; Fig. 5).

An ANOVA completed on the social structure of the new nests originating from multifemale versus solitary female parent nests produced a significant whole model test result ($F_{7,125} = 23.5$, $P < 0.0001$). As noted for dispersal distance, a significant source population effect was responsible for the significant whole model test result ($F_{3,129} = 22.6$, $P < 0.0001$). This significant source effect reflected the tendency for dispersing juveniles to express the social structure of the parental nest, regardless of the warm- or cold-water environment of the transplant site (Fig. 6). The frequency data presented in Fig. 6 show that multifemale nests were derived only from multifemale parental nests, which all originated from the cold-water sites, CL and MH.

Latitudinal Variation in Nest Integrity

Over the 3-month census period, focal nests and neighbouring nests coalesced at a frequency of

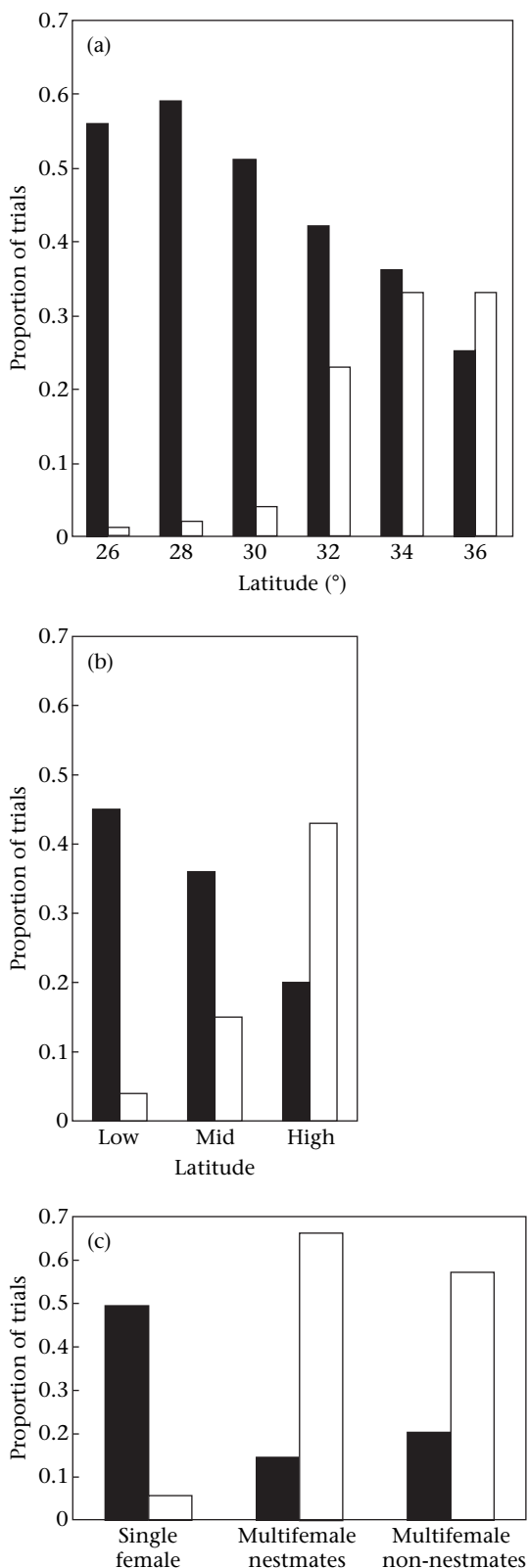


Figure 4. Behavioural phenotypes shown by adult female *A. studiosus* in interaction trials. □: 'attracted' (same corner) refers to a communal/cooperative social structure; ■: 'repelled' (opposite corner) refers to a solitary/territorial social structure. (a) Field-collected adult females with two sites within each latitude combined; (b) laboratory-reared F1 offspring of brooding females collected from low

approximately 25% at all sites for latitudes of 32° and above. No nest coalescence was observed below this latitude.

DISCUSSION

Every approach taken in this study to examine the social structure of *A. studiosus* corroborated the hypothesis that social polymorphism is characteristic of this species.

Evidence for Cryptic Species Lacking

Breeding experiment results provided no evidence for the existence of cryptic sister species, one solitary and the other social. The rate of successful matings in between-population crosses of near and far latitudes was equal to or greater than those observed for within-population pairings. In addition, crosses of individuals from sites separated by 4° and 10° latitude, respectively, were equally viable to clutches produced by within-population matings from each of the six latitudes (26–36°) examined. These results are consistent with the recent molecular analyses completed on species complexes in the genus *Anelosimus* and other members of the family Theridiidae: *A. studiosus* ranges from South America to North America (Agnarsson 2006).

Latitudinal Pattern of Social Structure

The volume of an *A. studiosus* nest is a first approximation of the relative frequencies of asocial (solitary/territorial nests) versus social (multifemale/communal nests) phenotypes. Nest censuses completed at replicated sites at 2° changes in latitude between south Florida (26°) and east Tennessee (36°) indicated that the dominant social structure is a solitary female nest. Small-scale variation in the size (i.e. volume) of solitary female nests is associated with the silk contribution that juveniles make to the natal nest (Jones & Parker 2000). Although the silk that juvenile *A. studiosus* contribute to a nest may help protect the nest from predation by foreign spiders (e.g. Anyphaenidae, genus *Anyphaena*; Perkins et al. 2007), the number of adult females present contributes more to silk volume than does the number of juveniles present. In the present study, both nest volume and the number of cooperating females within multifemale nests increased with latitude from the first appearance of multifemale nests at 30°.

Interaction trials provide a more detailed understanding of the latitudinal shift from populations consisting totally of solitary nests at low latitudes to a mix of solitary and multifemale nests at higher latitudes. Both phenotypes,

(26–28°), mid (30–32°) and high (34–36°) latitudes; (c) different trial contexts for adult females collected from sites in which multifemale nests were found: interactions between females from single-female nests (single female), from same multifemale nest (multifemale nestmates) and from multifemale nests collected from different sites (multifemale non-nestmates).

Table 2. Laboratory mating results within and between latitudes for the spider *Anelosimus studiosus*

Class	N	Proportion mating	Proportion with viable clutches	Mean±SE number of offspring	Proportion of offspring surviving
Within latitudes					
26°	17	0.59	0.90	10.5±3.4	0.60±0.11
28°	89	0.51	0.91	17.7±2.1	0.68±0.07
30°	55	0.71	1.0	13.3±2.0	0.61±0.06
32°	70	0.71	0.98	14.8±1.9	0.65±0.06
34°	103	0.55	0.88	14.6±1.9	0.64±0.06
36°	58	0.55	0.88	16.9±2.3	0.58±0.07
Between latitudes					
Near (4° diff)	24	0.76*	0.80	19.9±2.3	0.74±0.06
Far (10° diff)	51	0.83*	0.95	19.0±2.0	0.81±0.07
Test results		Chi-square $\chi^2 = 21.2$, $P < 0.05$	Chi-square $\chi^2 = 10.8$, NS	ANOVA $F_{7,263} = 1.6$, NS	ANOVA $F_{7,263} = 1.1$, NS

*Tests for directional effects in cross-population matings were nonsignificant.

asocial and social, were represented within local populations from 26° to 36°. However, the frequency of representation of the social phenotype varied markedly from about 1% at 26° to 33% of the individuals tested at 36°. In studies completed on the ecological correlates of *A. studiosus* social structure, we (Jones et al. 2007; T. C. Jones & S. E. Riechert, unpublished data) identified selection pressures against the social phenotype at warmer sites. At low frequencies of expression, individuals possessing the social phenotype will further have the problem of encountering other individuals and mates of the same phenotype.

Expression of Social Behaviour

Our scoring in the interaction trials for field-collected and F1 laboratory-reared individuals was conservative in

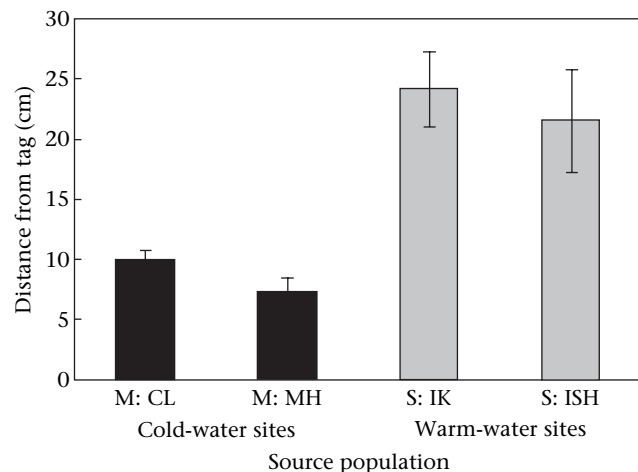


Figure 5. Means ± SE of dispersal distances (cm) of *A. studiosus* from tagged nests transplanted to new sites in cold-water and warm-water localities in east Tennessee. Nest composition at the time of transplant consisted of late-instar juveniles. X axis label refers to origin of dispersers: M = transplants of multifemale nest origin; S = transplants of single-female nest origin. CL (Chilhowee) and MH (Melton Hill) were cold-water source sites that had high frequencies of multifemale nests; IK (IC King) and ISH (Ish) were warm-water source sites that had only single-female nests.

that we assigned each trial result as belonging to one of the extremes of what might be a continuum of social behaviour, asocial versus social. Individuals of neither phenotype discriminate between nestmates and foreign *A. studiosus*: the asocial phenotype is repelled from all other adult females, while the social phenotype is attracted to them. This lack of discrimination of kin from nonkin seems to be a common characteristic of the communal/cooperatively social spiders and is also observed in diverse arthropod groups (e.g. see Costa 2006). The studies completed on communal spider associations, at least, suggest that colony members are almost exclusively kin (e.g. reviewed by Riechert & Roeloffs 1993), perhaps explaining the lack of selection for a discriminating mechanism. Social *A. studiosus* populations are viscous: individuals disperse only a few to 10s of centimetres from the parental nest if they disperse at all. Thus the probability of a foreign individual joining a nest seems very low. Ongoing molecular genetic studies will test the hypothesis that multifemale colonies in this spider also consist of close kin.

Based on the result of field censuses, we might have scored a third phenotype in the interaction trials that would be intermediate between asocial and social categories, 'tolerant'. Although we did not find multifemale nests below 32°, we did find closely adjoining solitary female nests and sometimes even clusters of nests with interconnecting silk threads. Increased tolerance of nearest neighbours is characteristic of spider species classified as colonial (Uetz & Hieber 1997; Whitehouse & Lubin 2005), where individuals form aggregations and often share a common silk framework. However, individual webs are maintained and there can be competition for favourable positioning within the aggregation. We did not include this third phenotype because an individual expressing it could be masked in the interaction trials where, for example, only one of the two individuals settling close to another was attracted to that individual. The second individual might merely have been tolerant of the individual that settled near it and have no propensity for cooperative behaviour. Additional field studies and breeding experiments are planned that will determine whether social phenotype is a quantitative trait showing

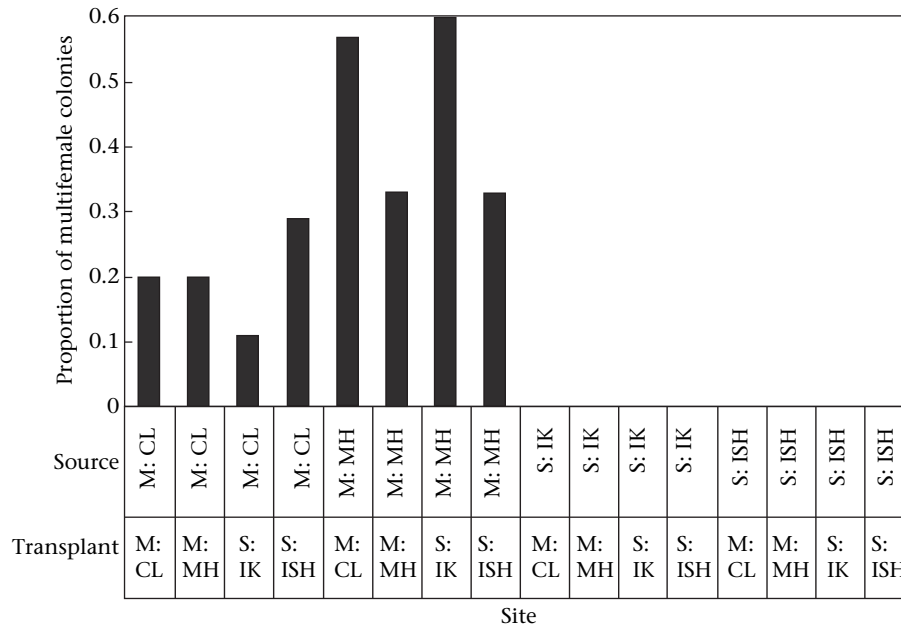


Figure 6. Results of transplant experiment. Bars refer to the distribution of multifemale nests in the vicinity of tagged transplant locations following dispersal from transplanted nests and the maturation of individuals the following spring. The source sites from which spiders were collected are listed on the top row of the X axis label and the sites to which nests were transplanted are listed on the bottom row. M = multifemale nest, cold-water sites: CL (Chilhowee Lake) and MH (Melton Hill); S = single-female nest, warm-water sites: IK (IC King Park) and ISH (Ish Creek).

a continuous distribution or a two- or three-phenotype polymorphism.

Several lines of evidence support the view that social behaviour in *A. studiosus* is, indeed, canalized in the sense that the behaviour of individuals is resistant to environmentally induced change (see review of plasticity and canalization by Debat & David 2001). From the interaction trials, for instance, we established that the distribution of behavioural types of F1 laboratory-reared offspring closely corresponded to that of field-collected individuals. The transplant experiment results also demonstrated a lack of individual plasticity. While warm-water sites in Tennessee favour single-female nests (Jones et al. 2007), multifemale nests transplanted to warm-water sites significantly produced new multifemale nests following dispersal at these sites. Furthermore, regardless of the site (cold or warm water) to which the reconstituted nests were transplanted, only individuals originating from multifemale nests produced multifemale nests. We have initiated fostering experiments that will provide a further test of the degree to which social behaviour in *A. studiosus* is canalized versus plastic.

Our finding that social behaviour appears to be a canalized trait in *A. studiosus* is in agreement with other studies that have examined the mechanisms underlying variable social behaviour in spiders (i.e. population variation in territory size in *Agelenopsis aperta* (Araneae: Agelenidae): Riechert 1979; Riechert & Maynard Smith 1989; population variation from territorial to colonial social structures in *Metepeira spinipes* (Araneae: Araneidae): Uetz & Cangioli 1986). While plasticity in social behaviour seems to be prominent among vertebrates (Ross & Keller 1995; Maher

& Lott 2000) and social insects, recent molecular studies are bringing attention to the existence of social trait canalization in these groups as well. Richards et al. (2003), for instance, identified the genetic basis underlying within-population variation in social structure of the sweat bee, *Halictus sexcinctus* (Fabricius) in southern Greece. Colonies within a population were either cooperative/communal or eusocial with morphological and behavioural castes present. DNA evidence was used to falsify the hypothesis that communal and eusocial forms are merely cryptic sister species. Molecular studies have also identified the specific regions of DNA linked to variable social behaviour (e.g. fire ant, *Solenopsis invicta*: Ross & Keller (1998); voles, *Microtus* sp.: Hammock & Young 2002, 2005).

Alexander (1974) defined social groups in terms of the genetic relationships existing among them, ranging from totally unrelated, as in bird flocks where group composition is continually changing, to genetically identical, as is the case in coelenterate colonies where individuals function as organs. Genetic relatedness studies using microsatellites are planned to determine the placement of *A. studiosus* multifemale colonies in this continuum, although the data presented here suggest that the majority of females in a multifemale colony may be sibs. First, dispersal distances measured during the course of the transplant experiments were much shorter for juveniles dispersing from multifemale colonies than for those dispersing from solitary female nests. Second, the coalescence of nests during the course of temporal checks of focal nests was observed only at the higher latitudes in sites where multifemale nests are found (coalescence

may occur through dragline following in this species; Furey 1998). Together, these results suggest that multifemale nests are composed largely although not necessarily exclusively of sibs.

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