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Amplified fragment length polymorphism fingerprints support limited gene flow among social spider populations

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We used DNA fingerprints to determine whether the population structure and colony composition of the cooperative social spider *Stegodyphus dumicola* are compatible with requirements of interdemic ('group') selection: differential proliferation of demes or groups and limited gene flow among groups. To investigate gene flow among groups, spiders were collected from nests at 21 collection sites in Namibia. Analysis of molecular variance showed a small but highly significant differentiation among geographic regions ($\Phi_{PT} = 0.23$, $P = 0.001$). Thirty-three nests at four collection sites (6–10 spiders per nest, 292 individual spiders) were investigated in more detail to evaluate variation within and among colonies and among collection sites. In these 33 nests, an average of 15% of loci (fingerprint bands) were polymorphic among nestmates; 16% of observed variance was partitioned among collection sites, 48% among nests within a collection site, and 36% among individuals within nests. Spatial autocorrelation analyses of spiders at three collection sites showed that the maximum extent of detectable spatial autocorrelation among individuals was approximately 30 m, indicating dispersal over greater distances is not typical. These results indicate limited gene flow among nests, as well as spatial structuring at the level of regions, local populations, and nests, compatible with interdemic selection. *Biological Journal of the Linnean Society*, 2009, **97**, 235–246.

ADDITIONAL KEYWORDS: AMOVA – dispersal – inbreeding – interdemic selection – population structure – social evolution – *Stegodyphus dumicola*.

INTRODUCTION

The cooperative or nonterritorial permanently-social spiders (Aviles, 1997; Lubin & Bilde, 2007) live in colonies containing one or several nests with a few to thousands of spiders. They are characterized by a female-biased sex ratio, an absence of dispersal by immature stages, and mating among nest-mates. Social spiders comprise fewer than 25 species (Buskirk, 1981; Aviles, 1997; Agnarsson *et al.*, 2006), but their unusual social system and population structure evolved independently at least 18 times (Agnarsson *et al.*, 2006; Lubin & Bilde, 2007). Social spiders have been proposed as model systems for the evolution of cooperation by means of group or interdemic selection (Smith & Hagen, 1996; Lubin & Bilde, 2007). Interdemic selection requires populations that are subdivided into groups, limited migration among

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groups, frequent extinction of groups, and differences among groups in the production of propagules or 'daughter' groups (Aviles, 1993; Lubin & Bilde, 2007). Some of these conditions appear to be met by social spiders.

Social spider populations are highly subdivided. Spider societies live in discrete nests or colonies; juvenile dispersal is absent and mating occurs among nest mates (Aviles, 1997; Lubin & Bilde, 2007). Colonies give rise to daughter colonies through dispersal of inseminated females or by colony fission, which creates clusters of related colonies originating from the same maternal lineage (Seibt & Wickler, 1988; Henschel, 1998; Schneider *et al.*, 2001). Colony extinction rates are high, and larger colonies have a higher probability of survival and subsequent reproduction (*Anelosimus eximius* Theridiidae: Christenson, 1984; Aviles & Trufino, 1998; *Agelena consociata* Agelenidae: Riechert, Roeloffs & Echternacht, 1986; *Stegodyphus dumicola* Eresidae: Bilde *et al.*, 2007). The highly female-biased sex ratio characteristic of social spiders has been proposed to be favoured by interdemic selection because it can contribute to rapid colony growth and hence a higher probability of survival and colony proliferation (Aviles, 1986, 1993; Smith & Hagen, 1996; Aviles *et al.*, 2000).

Stegodyphus dumicola is an excellent subject for the study of behaviour, population structure, and gene flow among social spider colonies (Kraus & Kraus, 1988). Nests of *S. dumicola* in southern Africa are found primarily on low acacia (thornbush) shrubs and trees in savanna and grassland habitats and may be clustered in patches. A large nest with females and young can contain several hundred individuals, but more typical nest sizes before hatching of the young comprise fewer than 20 females (Henschel, 1991/1992; Henschel, 1998; Lubin & Bilde, 2007).

The turnover rate of *S. dumicola* colonies is high (Lubin & Crouch, 2003), probably due to intracolony competition when colonies exceed optimal colony size, or to environmental factors (Lubin & Bilde, 2007). Hence, the traits described result in differential proliferation rates (production of daughter colonies) among colonies, such that selection may operate at the level of the deme or group.

Several methods of nest foundation and dispersal are documented in *S. dumicola*. Mated adult females were observed dispersing from large nests by walking or bridging over short distances and by ballooning, which may be a means of both short and long-distance dispersal (Schneider *et al.*, 2001). Females were also observed joining the incipient nest of another female (Henschel, 1991/1992). Nests can undergo fission to produce daughter nests, which sometimes maintain silk connections between them. Males may move short distances to adjacent nests (Lubin *et al.*, 2009). However, the relative frequencies of each method of nest initiation and the extent of dispersal among nests are not known. Does gene flow or migration among nests take place? If so, does it occur via dispersal of males or females and how far do they disperse? A more detailed understanding of population genetic structure and dispersal could be used to examine whether social spiders truly conform to the requirements for interdemic selection.

Earlier genetic studies of social spiders indicated low levels of genetic variation and little gene flow among colonies. Mitochondrial (mt)DNA sequence data suggested that colonies of *S. dumicola* most often originate from a single maternal lineage (Johannesen *et al.*, 2002), although approximately 13% of colonies contained more than one mtDNA haplotype, which could result from dispersing females joining existing nests or unrelated dispersing females joining to initiate a new nest (Johannesen *et al.*, 2002). Allozyme variation appears to be substantially lower in social species than in subsocial congeners (Smith, 1987; Johannesen & Lubin, 2001). In four social species from four different families, polymorphic allozyme loci are rare (0–32% of loci examined), and colony mates are usually identically homozygous (*Achearanaea wau* Theridiidae: Lubin & Crozier, 1985; *Anelosimus eximius* Theridiidae: Smith, 1986; Smith & Hagen, 1996; *Agelena consociata* Agelenidae: Roeloffs & Riechert, 1988; *Stegodyphus sarasinorum* Eresidae: Smith & Engel, 1994]. However, the very fact that allozyme variation is low in cooperative spiders means that there are very few markers to study variation within and among nests or to track dispersal. In the present study, we apply a modified amplified fragment length polymorphism (AFLP) fingerprinting technique (Vos *et al.*, 1995), three-enzyme (TE)-AFLP (van der Wurff *et al.*, 2000), to investigate dispersal and intra- and intercolony genetic variation. AFLPs and TE-AFLPs require no prior knowledge of the organism's genome, sample variation at many loci across the genome simultaneously, and typically reveal substantially more polymorphisms than allozymes.

MATERIAL AND METHODS

COLLECTIONS

Intact nests of *S. dumicola* were collected in 1993 by JRH, from 21 locations in Namibia (Fig. 1, Table 1). Latitude and longitude were recorded for each site. All spiders were stored frozen at –80 °C. Only females or very large juveniles were used to ensure that all nest mates compared were members of the same generation, and not parents and offspring.

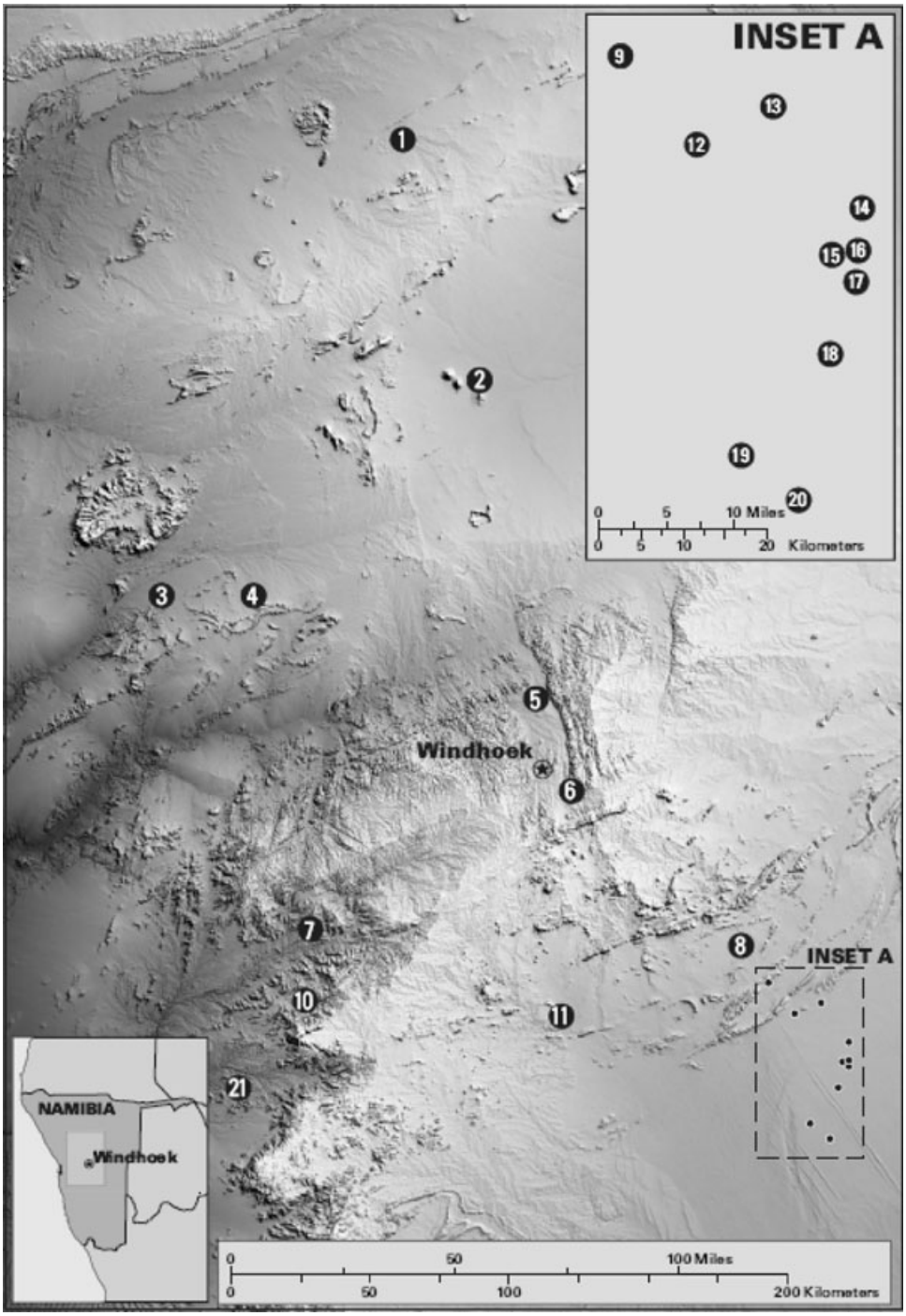

Figure 1. Map of *Stegodyphus dumicola* collection sites in Namibia. Numbers correspond to those shown in Table 1. Colonies at site 12 (Nauas), 15–16 (Christirina house and farm), 17 (Tivoli), and 20 (Uhlenhorst) were examined for variation within and among colonies; the relative positions of colonies at sites 15–16, 17, and 20 were mapped and used in spatial autocorrelation analyses.

Sampling and analysis

To investigate regional patterns of genetic variation, we sampled one spider from each of 44 nests from 21 collection sites (Fig. 1, Table 1). To examine genetic variation within and among nests and among collection sites, we focused on four sites (Christirina, Nauas, Tivoli, and Uhlenhorst) from which multiple nests containing large numbers of spiders were col-

Table 1. Namibian collection sites for *Stegodyphus dumicola* used in study of large-scale geographic variation, showing number of nests sampled at each site, and group (A, B or C) into which each collection site was placed based on geographic proximity

Collection site	Latitude	Longitude	Date	Number of nests
Group A: polymorphic loci = 69.6%; H_E (SE) = 0.24 (0.04)				
1. Otjitasu	20°26′S	16°34′E	May to June 1993	1
2. Omatako Hills	21°13′S	16°49′E	May to June 1993	1
3. Karibib	21°55′S	15°47′E	March to April 1993	1
4. Albrechtshohe	21°55′S	16°05′E	March to April 1993	1
Group B: polymorphic loci = 43.5%; H_E (SE) = 0.17 (0.04)				
5. Teufelsbach	22°15′S	17°00′E	March to April 1993	1
6. Windhoek – E	22°33′S	17°07′E	March to April 1993	1
7. Kiamsab	23°00′S	16°16′E	March 1993	1
10. Djab Farm	23°14′S	16°15′E	March to April 1993	1
11. Rehoboth	23°17′S	17°05′E	May to June 1993	1
21. Arizona	23°31′S	16°02′E	May to June 1993	1
Group C: Polymorphic loci 56.5%; H_E (SE) = 0.18 (0.04)				
8. Ibenstein Farm	23°00′S	17°40′E	March to April 1993	1
9. Tigerpforte	23°13′S	17°45′E	March to April 1993	1
12. Nauas	23°17′S	17°50′E	March to April 1993	4
13. Kitripotip	23°17′S	17°55E′	March to April 1993	1
14. Dornenpfanne	23°21–23′S	18°01′E	March to April 1993	1
15–16. Christirina	23°24–26′S	18°01′E	March to April 1993	22
17. Tivoli	23°26′13″S	18°0′34″E	May to June 1993	1
18. Rheinpfalz	23°32–33′S	17°59′E	Mar to Apr 1993	1
19. Kaukerus	23°37′30″S	17°53′E	May to June 1993	1
20. Uhlenhorst	23°40′44″S	17°57′	May to June 1993	1

Collection site numbers correspond to sites in Fig. 1.

lected. At these four sites, we sampled a total of 292 spiders from 33 nests. The relative positions of nests at the Christirina, Uhlenhorst, and Tivoli sites were mapped, enabling us to analyse genetic similarity among nests as a function of distance (see below).

DNA FINGERPRINTING

DNA was extracted from entire spiders or from legs using DNEasy (Qiagen) or Sigma's GenElute Mammalian Genomic DNA Miniprep kits in accordance with the manufacturer's protocols. Aliquots of each DNA extract were digested with the six-base restriction enzymes *Xba*I and *Bam*HI and the four-base enzyme *Rsa*I. The resulting fragments were ligated to linkers (van der Wurff *et al.*, 2000) with sticky ends complementary to the *Xba*I and *Bam*HI sticky ends of the DNA fragments. '*Xba*I-CC' and '*Bam*HI-C' primers described in van der Wurff *et al.* (2000) were used to amplify a subset of the fragments. These primer sequences were identical to one strand of each linker, with arbitrary extensions -CC and -C, respectively. Each 20-μL digestion/ligation reaction contained: 2 μL of 10× ligation buffer (Promega), 2 μL of 500 mM NaCl, 4 μL of *Bam*HI adaptor at 1 pmol μL^{-1}, 4 μL of *Xba*I adaptor at 1 pmol μL^{-1}, 0.6 μL of ligase at 3 U μL^{-1}, 0.125 μL of *Bam*HI at 10 U μL^{-1}, 0.6 μL of *Xba*I at 10 U μL^{-1}, 0.1 μL of *Rsa*I at 10 U μL^{-1}, 2 μL of DNA extract, and 4.5 μL of water (buffers and enzymes supplied by Promega and New England Biolabs). Reactions were incubated at 30 °C for 1.5 h, and then stored at –20 °C.

A subset of the ligated DNA fragments was amplified by the polymerase chain reaction (PCR). Each 12.75 μL of PCR reaction contained: 1.25 μL of 10 × PCR buffer, 0.75 μL of 25 mM $MgCl_2$, 0.25 uL (2.5 pmol) of *Bam*HI-C primer, 0.25 μL (2.5 pmol) of *Xba*I-CC primer end-labelled with ^{33}P or ^{32}P, 0.125 μL of *Taq* polymerase at 5 U μL^{-1}, 0.125 μL of dNTPs (polymerase mix, 20 mM each dNTP), 0.5 μL of digested, ligated DNA, and 9.5 μL of water (buffers and enzymes supplied by Promega; dNTPs by Gibco BRL). The thermal profile for amplification, as described by van der Wurff *et al.* (2000), comprised: 3 min denaturation at 95 °C; followed by 95 °C for 30 s, 70 °C for 30 s, and 72 °C for 60 s for ten cycles;

95 °C for 30 s, 60 °C for 30 s, and 72 °C for 60 s for 40 cycles; and, finally, 72 °C for 20 min.

Reactions were electrophoresed through 8% denaturing polyacrylamide/TBE gels for 5 h at 1800 V. Gels were dried on filter paper and used to expose Fuji X-ray film overnight. The repeatability of the method was demonstrated by duplicating some individuals on multiple gels; this also provided an easy way to homologize bands across gels. Bands were scored by hand as present or absent for each individual. Thus each individual spider was characterized by a composite AFLP 'phenotype' consisting of the state (present or absent) of each of the bands scored in these samples.

Data were analysed using the Microsoft Excel add-in program Genetic Analyses in Excel, version 6 (GenAlEx6: Peakall & Smouse, 2006). For each set of samples and for each population (for a definition of populations, see below), we identified unique banding patterns or phenotypes and calculated percent polymorphic loci and mean expected heterozygosity (see below).

Estimation of H_E followed the method of Lynch & Milligan (1994), which considers each band position as a different locus with two alleles: 'band amplified' (dominant) and 'band not amplified' (recessive); the absence of a band indicates a recessive homozygote. Populations of sexually reproducing organisms are assumed to be in Hardy–Weinberg equilibrium. Although we hypothesize that *S. dumicola* societies are inbreeding, in the sense that all or most mating takes place among nest mates, we expect that within nests individuals mate randomly. An earlier allozyme study of another cooperative social species, *Anelosimus eximius* (Smith & Hagen, 1996) indicated that genotype frequencies within colonies conformed to Hardy–Weinberg expectations.

At each locus, the frequency of the recessive allele (q) is estimated from the frequency of putative recessive homozygotes (q^2), and the frequency of the dominant allele is estimated as: $p = 1 - q$. Expected heterozygosity at each locus, $h = 1 - \Sigma x_i^2$, where x_i is the frequency of the ith allele, or in this case, $1 - (p^2 + q^2)$. Expected heterozygosity averaged over all loci was calculated as:

$$H_E = 1 - 1/m \sum_{y=1}^{m} \sum x_i^2$$

where y represents loci or bands 1 through m.

Analysis of molecular variance (AMOVA) (Excoffier, Smouse & Quattro, 1992) implemented in GenAlEx6 was used to assess the patterns of observed genetic variation at the regional, local (collection site) and nest level. AMOVA calculates statistics analogous to Wright's F-statistics (Wright, 1951, 1965). Φ_{PT}, which can be in the range 0–1, measures the similarity of pairs of individuals drawn at random from the same population, relative to pairs of individuals drawn from the total sample (analogous to F_{ST}). Φ_{RT} measures the similarity between pairs of individuals drawn at random from the same region or group of populations compared to individuals drawn at random from the total sample. Φ_{PR} is the correlation among individuals drawn at random from the same population compared to individuals drawn at random from populations in the same region.

Regional differentiation

The 44 nests from 21 collection sites were grouped into three regions based on geographic proximity (Fig. 1, Table 1). AMOVA was used to estimate genetic differentiation within and among the populations. To test for significance, Φ_{PT} values calculated from observed data were compared to a distribution of values obtained from 999 permutations assigning individuals randomly to populations of the same sizes. Although most sites were represented by a single nest, multiple nests were collected from two closely-spaced sites (Christirina, 22 nests; Nauas, 4 nests). Because these two sites could exert a strong influence on the significance of groupings, we also performed a set of 88 AMOVAs using all combinations of one nest each from Nauas and Christirina. A spatial autocorrelation analysis (see below) investigated relatedness among individuals as a function of distance.

Differentiation within and among nests and collection sites

The second analysis focused on four sites (i.e. Nauas, Tivoli, Uhlenhorst, and Christirina) from which large numbers of nests were collected. Thirty-three nests containing multiple adults or large juveniles were selected for study. Six to ten spiders were sampled per nest for a total of 292 spiders (this analysis did not include the 44 spiders used in the regional analysis above). Individual spiders were grouped by nest, and nests were grouped by collection site. AMOVA was used to assess variation among collection sites (Φ_{RT}), among nests within collection sites (Φ_{PR}), and among individuals within nests (Φ_{PT}). Significance was determined as above, by comparison of Φ statistics calculated from the observed data to the distribution of values generated by 999 permutations of the data into groups of the same sizes.

Nests at three sites (i.e. Christirina, Tivoli, and Uhlenhorst) were mapped on grids so that distances among pairs of nests could be measured. Relatedness as a function of distance between nests was investigated using spatial autocorrelation implemented under the 'Spatial' menu in GenAlEx. The 'Multipops' option analysed data from each collection site

individually and in a combined analysis, using the variable distance class option. These procedures require as input a matrix of pairwise squared genetic distances among individuals and a matrix of pairwise geographic distances among individuals. Distances among colonies varied greatly among collection sites: 2–383 m at Christirina, 2.7–41 m at Uhlenhorst, and 895–6832 m at Tivoli. Distances for the analyses were chosen after inspection of the pairwise geographic distance matrices for the three populations to capture the range of distances among colonies, while avoiding distance classes with few data points (Smouse & Peakall, 1999; Peakall, Ruibal & Lindenmayer, 2003; Peakall & Smouse, 2006). The distance 0.1 m corresponds to individuals from the same colony. The output is a correlation coefficient, *r*, which can take values from –1 to +1; *r* provides a measure of the genetic similarity between pairs of individuals separated by specified distances.

Significance testing was carried out through both permutation and bootstrapping approaches. In each of 1000 permutations, individuals were randomly assigned to geographic locations; for each permutation, a correlation coefficient, *rp*, was calculated and the 1000 *rp* values were ranked. The 25th and 975th ranked values of *rp* were taken as upper and lower boundaries of 95% confidence interval under the assumption of no geographic structure. In a two-tailed test, *r* is considered significant if it falls outside the 95% confidence interval. In the present study, we used a one-tailed test because we had reason to expect positive values for the correlation coefficient due to restricted dispersal. The computed *r* value was compared to the 1000 *rp* values obtained by permutations to determine the probability of obtaining a value as large as or larger than the calculated *r* under the null hypotheses of no geographic structure. A confidence interval around our calculated value of *r* was obtained by bootstrapping individuals within distance classes.

RESULTS

Regional variation

The presence or absence of 23 bands was scored for each of 44 spiders from 21 sites. Of these, one band was fixed (present in all individuals) and 22 (95%) were polymorphic (present in at least one individual *and* absent in at least one individual). The 22 polymorphic bands produced 35 unique banding patterns or phenotypes. Pairwise distance among phenotypes was in the range 1–13. Mean ± SE expected heterozygosity (H_E) was 0.26 ± 0.04. Considering the three regional groups of collection sites, Group A included four sites, four spiders, and four phenotypes; Group B included six sites, six spiders, and five phenotypes; and Group C included ten sites, 34 spiders, and 26 phenotypes. Percent polymorphic loci and H_E for each group are shown in Table 1. Both AMOVA analyses (i.e. that using all nests and that using only one nest per collection site) showed significant genetic differentiation among these regions (Table 2). For the more conservative analyses using one nest per collection site (including all 88 combinations of the 22 nests from Christirina and four nests from Nauas), the mean value of Φ_{PT} was 0.310 (median = 0.310, mode = 0.320, range 0.29–0.34, probability of a value as great or greater = 0.001–0.003), and 31% of

Table 2. Large scale regional differentiation in *Stegodyphus dumicola*

	Analysis 1: one spider per nest, all nests	Analysis 2: one spider per nest, one nest per collection site
Number of samples	44 nests	20 nests
Number of populations	3	3
Number of regions	1	1
Variance		
Among populations	38%	29–34%
Within populations	62%	66–71%
Φ_{PT} (p)	0.38 ($P = 0.001$)	Mean 0.31 ($P = 0.001$)
H_E over all nests	0.24 (0.035)	

Nests were assigned to one of three groups based on geographic proximity (Fig. 1, Table 1). Analysis of molecular variance (Excoffier *et al.*, 1992; Peakall & Smouse, 2006) was used to examine differentiation among groups of nests. To test for significance, 999 permutations were performed comparing population subdivision observed in each of the proposed groupings to that obtained when individuals were assigned at random to groups of similar size. Analysis 1 includes 44 nests, including 22 from Christirina and four from Nauas. In analysis 2, only one nest was used per site, but 88 repetitions were performed using all possible combinations of one nest each from Nauas and Christirina.

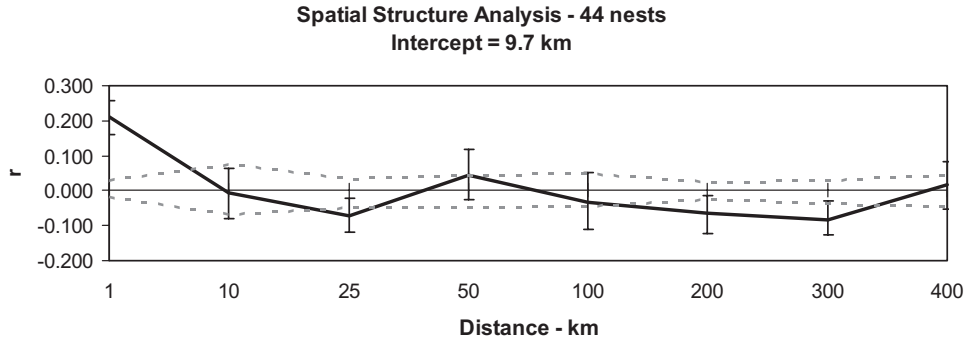

Figure 2. Spatial autocorrelogram for 44 nests of *Stegodyphus dumicola* collected from 20 sites. Dashed lines indicate the upper and lower boundaries of 95% confidence interval for values of r under the null hypothesis of no geographic structure. Solid line connects r values for each distance class. Bars around computed r values are 95% bootstrap confidence intervals. The autocorrelation coefficient r for the distance class '≤ 1 km' = 0.21 ($P < 0.001$). This is the only distance class for which significant geographic structure was detected and corresponds to nests at the same collection sites (Nauas and Christirina).

observed variance was partitioned among the regions. Significant spatial autocorrelation was detected only over distances of 1 km or less, corresponding to nests at the same collection site (Fig. 2).

Variation within and among nests

The presence or absence of 28 bands was scored for each of 292 spiders from 33 nests from the four sites from which multiple nests were collected (Christirina, Nauas, Tivoli and Uhlenhorst; Table 3). Of these, eight bands were fixed and 20 (71.4%) were polymorphic. The 20 polymorphic bands produced 119 unique phenotypes with pairwise distances among phenotypes in the range 1–12. Mean ± SE expected heterozygosity, H_E, for all individuals combined was 0.195 ± 0.034.

Overall, 52 phenotypes were found among the 77 spiders sampled from Christirina, 69 phenotypes among 117 spiders from Nauas, 24 phenotypes among 63 spiders from Tivoli, and 18 phenotypes among 35 spiders from Uhlenhorst. The number of phenotypes observed per nest was in the range 1–10 (mean ± SE per nest = 4.9 ± 2.5). Within nests, percent polymorphic loci was in the range 0–39.3% (mean 15.3%). Expected heterozygosity within nests was in the range 0–0.144 (Table 3). Three nests (one each from Nauas, Tivoli, and Uhlenhorst) were monomorphic, consisting of spiders with the same phenotype. In three other nests from the same populations, only two phenotypes were present. In these nests, the two phenotypes differed by the presence of only one (Nauas and Tivoli) or two (Uhlenhorst) bands, and the rarer phenotype was detected in only one nest-mate.

AMOVA statistics show significant genetic differentiation among colonies, as well as differentiation among the four collection sites (Table 4). Within each collection site, differentiation among nests was surprisingly high: Christirina, $\Phi_{PT} = 0.50$ ($P = 0.01$); Nauas, $\Phi_{PT} = 0.54$ ($P = 0.01$); Tivoli, $\Phi_{PT} = 0.75$ ($P = 0.001$); and Uhlenhorst, $\Phi_{PT} = 0.44$ ($P = 0.01$).

Results of spatial analyses of Christirina, Tivoli, and Uhlenhorst populations (i.e. the only three populations for which map data were available) are shown in Figure 3. In all three sites, the highest values of the autocorrelation coefficient, r, occurred at distances of 0.1 m or less, corresponding to individuals in the same nest. Values of r are still significant at 2 m (except for Tivoli, which had no nests closer together than 895 m). In the combined analysis, values of r are significant at 0.1, 2, and 25 m. The x-intercept, which is interpreted as the maximum distance at which significant spatial autocorrelation is detected, is 32.6 m.

DISCUSSION

TE-AFLP fingerprinting proved to be much more successful than allozymes in detecting genetic variation in populations of *S. dumicola*: an earlier allozyme survey (D. Smith, unpubl. data) revealed no allozyme variation among samples from Namibia, whereas 71–95% of bands scored in DNA fingerprinting surveys were polymorphic. Although more variation is detected, the pattern of variation still conforms to that revealed by earlier allozyme studies of other cooperative social spiders: genetically similar nest mates, genetic differentiation among nests, and a smaller but statistically significant amount of differentiation among geographic regions and collection sites (Lubin & Crozier, 1985; Smith, 1986; Roeloffs & Riechert, 1988; Smith & Engel, 1994; Smith & Hagen, 1996).

Table 3. Genetic diversity and differentiation among *Stegodyphus dumicola* in four collection sites

Collection site	Nest code	Number spiders	Number phenotypes	Polymorphic bands	H_E
Christirina	CHR02	9	6	14.3%	0.056 (0.028)
$\Phi_{PT} = 0.50$	CHR07	9	3	7.1%	0.027 (0.019)
$P = 0.01$	CHR17	9	8	25.0%	0.064 (0.026)
	CHR24	7	6	14.3%	0.040 (0.021)
	CHR32	8	7	21.4%	0.094 (0.035)
	CHR36	9	5	21.4%	0.072 (0.031)
	CHR39	9	3	17.9%	0.077 (0.033)
	CHR44	9	6	10.7%	0.051 (0.028)
	CHR45	8	8	28.6%	0.100 (0.033)
		Total = 77	**Mean = 5.8**	**Mean = 17.9%**	
Nauas	NAU02	8	7	21.4%	0.081 (0.032)
$\Phi_{PT} = 0.54$	NAU03	10	9	21.4%	0.080 (0.031)
$P = 0.01$	NAU04	10	4	10.7%	0.025 (0.016)
	NAU05	9	9	32.1%	0.144 (0.041)
	NAU06	10	8	17.9%	0.081 (0.033)
	NAU09	10	10	39.3%	0.123 (0.035)
	NAU11	10	4	10.7%	0.014 (0.008)
	NAU14	10	5	10.7%	0.038 (0.021)
	NAU15	10	7	14.3%	0.055 (0.027)
	NAU18	10	1	0%	0
	NAU19	10	2	3.6%	0.007 (0.007)
	NAU20	10	3	10.7%	0.036 (0.021)
		Total = 117	**Mean = 5.8**	**Mean = 16.1%**	
Tivoli	TIV01	9	3	7.1%	0.034 (0.023)
$\Phi_{PT} = 0.75$	TIV03	9	5	17.9%	0.083 (0.034)
$P = 0.001$	TIV05	10	5	14.3%	0.068 (0.032)
	TIV06	10	4	17.9%	0.038 (0.020)
	TIV07	9	4	14.3%	0.047 (0.024)
	TIV08	10	1	0%	0
	TIV10	6	2	4.3%	0.006 (0.006)
		Total = 63	**Mean = 3.4**	**Mean = 12.26%**	
Uhlenhorst	UHL02	6	2	7.1%	0.21 (0.017)
$\Phi_{PT} = 0.44$	UHL06	7	1	0%	0
$P = 0.01$	UHL09	8	7	21.4%	0.067 (0.028
	UHL32	7	3	28.6%	0.094 (0.032)
	UHL35	7	4	14.3%	0.063 (0.03)
		Total = 35	**Mean = 3.4**	**Mean = 14.3%**	
All individuals		**Total 292**	**119**	**71.4%**	**0.195 (0.034)**

Φ_{PT} values (analysis of molecular variance; Excoffier *et al.*, 1992; Peakall & Smouse, 2006) indicate differentiation among nests within a collection site. Number of phenotypes, percent of three-enzyme-amplified fragment length polymorphism bands found to be polymorphic, and expected heterozygosity within each nest were calculated as described in text.

Table 4. Hierarchical partitioning of genetic variation among nests and collecting sites for *Stegodyphus dumicola*, using analysis of molecular variance (Excoffier *et al.*, 1992) implemented in Genalex6 (Peakall & Smouse, 2006)

	Φ statistics	Significance	% Molecular variance
Among individuals/within nests	$\Phi_{PT} = 0.640$	$P = 0.01$	36%
Among nests/within region	$\Phi_{PR} = 0.571$	$P = 0.001$	48%
Among regions	$\Phi_{CT} = 0.162$	$P = 0.001$	16%

Samples consist of 292 spiders, taken from 33 nests located in four collection sites (Table 3).

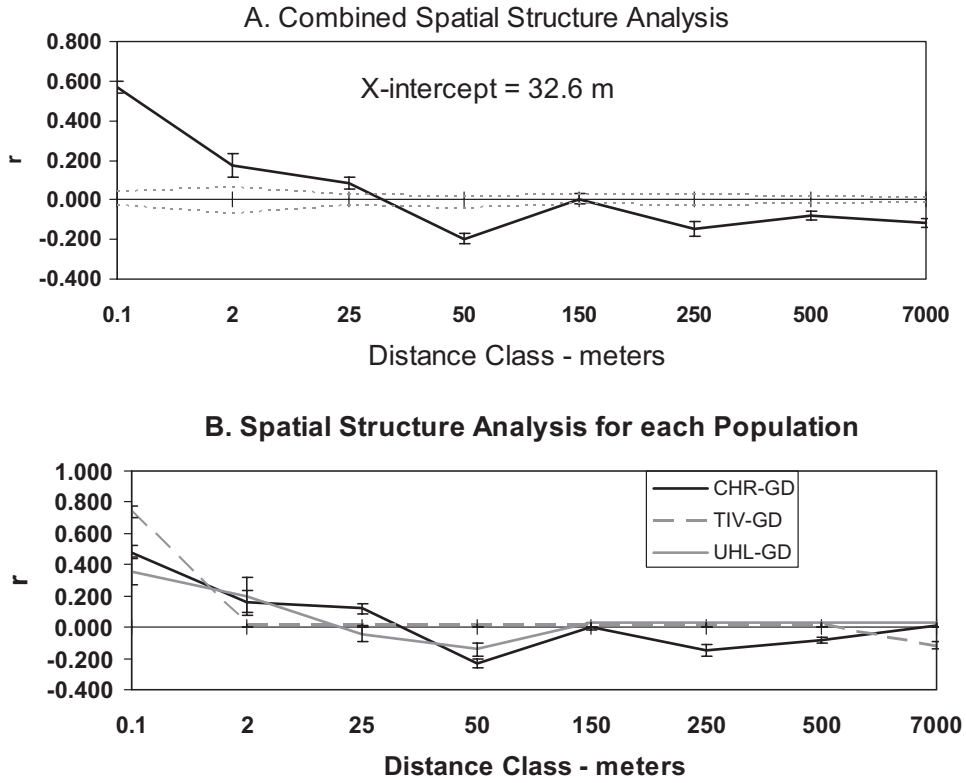

Figure 3. Spatial structure analyses for three *Stegodyphus dumicola* collection sites, Christirina, Uhlenhorst and Tivoli. A, combined analysis includes all samples. Dashed lines indicate upper and lower boundaries of 95% confidence interval for values of *r* under the null hypothesis of no geographic structure. Solid line connects *r* values for each distance class. Bars around computed *r* values are 95% bootstrap confidence intervals. B, analysis for three collection sites, Christirina (CHR-GD), Tivoli (TIV-GD), and Uhlenhorst (UHL-GD) calculated individually. Bars around computed *r* values are 95% bootstrap confidence intervals.

One important difference between the results of allozyme and fingerprinting studies concerns intracolony variation. Although allozyme studies of cooperative spiders typically reveal little or no polymorphism among nest-mates, TE-AFLPs sampled a greater portion of the genome and revealed genetic variation within most nests: an average of 15% of loci were polymorphic within nests, and 36% of observed variance occurred among individuals within nests.

If new nests were established by mated females or by groups of females from the same natal nest, and if all mating occurred exclusively among nest mates, then genetic drift within the small populations in nest lineages would result in loss of all or most intra-nest variation within a few generations. Polymorphism within nests indicates gene flow among nests or 'nest lineages' (daughter nests derived from pre-existing nests without gene flow from other nests). The level and pattern of intra-nest polymorphism revealed by the TE-AFLP data is in keeping with field observations indicating that dispersing *S. dumicola* males and females may join existing nests (Henschel, Lubin & Schneider, 1995). By contrast, in six of the 33 nests examined (18% of nests), nestmates were monomorphic or nearly monomorphic. These nests were similar to others at the same collection sites in terms of size and number of females and sub-adults observed (D. Smith & J. Henschel, unpubl. data). Such monomorphic nests may originate from single mated females or groups of sisters that have mated with male nestmates.

At the largest geographic scale, AMOVA analysis detected genetic differentiation among the three geographic regions ($\Phi_{PT} = 0.23$, $P = 0.001$; Table 2), but spatial autocorrelation analysis showed significant values of *r* only at distances of 1 km or less (Fig. 2). Because the only nests separated by 1–10 km or less were at the same collection site, spatial autocorrelation analysis at this scale is simply measuring genetic differentiation among collection sites.

AMOVA statistics for 33 nests from four collection sites revealed high levels of genetic differentiation among nests within collection sites (Φ_{PT} among nests within each of the four collection sites was in the

range 0.44–0.75; Table 3). Spatial autocorrelation and spatial structure analyses also showed that the highest correlations among phenotypes were among nest-mates (r among pairs of individuals in the same nests = 0.74 at Tivoli, 0.39 at Uhlenhorst, 0.48 at Christirina, and 0.58 for all three sites combined; Figs 3A, B). In the combined spatial analysis (Fig. 3A) and at Christirina, the site with the largest number of sampled nests, the x-intercept was 31–32.6 m. This indicates that, although migration and gene flow take place among nearby colonies, detectable relatedness among neighboring colonies drops off steeply with distance, and dispersal over distances greater than 30 m is not typical. Whether the movement among nearby nests is primarily by males, or due to dispersing females, cannot be determined with the data obtained in the present study (all specimens were females or large juveniles) (see companion paper by Lubin *et al.* (2009) on male dispersal in this issue). An analysis of population structure using males and females collected post-mating may answer this question (Peakall *et al.*, 2003).

These results complement a study of mtDNA sequence variation in *S. dumicola* populations in Namibia, which also revealed a surprising amount of diversity. In most nests, Johannesen *et al.* (2002) found a single mtDNA haplotype, consistent with nest initiation by one female or by sisters (mean number haplotypes per nest = 1.13). They also found a surprisingly high level of divergence among haplotypes: the 15 observed haplotypes fell into four lineages, with one to five substitutions among haplotypes within a lineage and 12–29 substitutions among lineages. Superficially, such high mitochondrial diversity appears to be at odds with the notion of limited gene flow among nests. The smaller effective population size of uniparentally inherited mitochondrial genomes (compared to nuclear genomes) should lead to more rapid loss of diversity (Birky, Maruyama & Fuerst, 1983, Ballard & Whitlock, 2004) and 'pruning' of diversity to single lineage. However, when the mtDNA and DNA fingerprinting data are considered together, they suggest a solution to this puzzle.

DNA fingerprinting and spatial autocorrelation analyses indicate that distances traveled by dispersing individuals are typically on the order of 30 m or less. The mtDNA data showed that, with the exception of one collection site, all nests at a collection site belonged to the same mtDNA lineage. These both indicate that long distance dispersal of females between regions is rare, and that a stepping-stone model is more consistent with *S. dumicola* dispersal. Long-term isolation of regions (by distance or other barriers) allows different mtDNA lineages to persist and diversify in each region. Within local populations (corresponding to our collection sites), colony foundation is typically by one female or by females sharing the same mtDNA genotype, although there may be movement of females from nest to nest within a collection site, as indicated by our TE-AFLP data and by the three nests observed by Johannesen *et al.* (2002), which contained two mtDNA haplotypes. Thus, *S. dumicola* populations are structured at several scales: the nest, the local population, and larger, regional populations, which may belong to different mtDNA lineages.

This structuring provides opportunities for both kin selection and interdemic selection. Interdemic selection requires populations that are subdivided into groups, limited migration among groups, and differences among groups in productivity, leading to frequent initiation and extinction of groups. *Stegodyphus dumicola* populations are subdivided into colonies (nests) composed of related individuals. New nests are initiated by mated females dispersing from previously established nests, as well as by fissioning of established nests. Migration among nests is limited, as indicated by the strong genetic differentiation among nests, and migration that does occur is typically over short distances, as demonstrated by the spatial autocorrelation data. Most nests last only a few years or less (Lubin & Crouch, 2003; Bilde *et al.*, 2007). Differences among nest lineages in the production and survival of daughter nests provide an opportunity for interdemic selection of traits that further enhance survival and reproduction of nest lineages. Female-biased primary sex ratio may be one such trait. Females of *S. dumicola* produce only a single brood; all females in the nest feed the young by regurgitation and the young spiderlings eventually kill and eat their mother as well as other nonreproducing females (Salomon & Lubin, 2007). Thus, colony growth is enhanced by the presence of more adult females. Another trait possibly promoted by interdemic selection is the tendency of social spiders to accept into their colonies individuals transplanted from other, sometimes distant nests (Seibt & Wickler, 1988). If additional spiders contribute positively to nest maintenance and survival, then acceptance of additional helpers may be favoured by interdemic selection. If nearby nests tend to contain close relatives, the joining spider may also increase its own inclusive fitness. The evidence provided in the present study for population structuring and limited gene flow among nests of the social *S. dumicola* supports the hypothesis that interdemic selection can contribute to the maintenance of sociality in spiders.

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