

## SHORT COMMUNICATION

**Low genetic diversity and strong but shallow population differentiation suggests genetic homogenization by metapopulation dynamics in a social spider**

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*Department of Bioscience, Aarhus University, Aarhus C, Denmark**Keywords:*

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gene flow;  
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**Abstract**

Mating systems and population dynamics influence genetic diversity and structure. Species that experience inbreeding and limited gene flow are expected to evolve isolated, divergent genetic lineages. Metapopulation dynamics with frequent extinctions and colonizations may, on the other hand, deplete and homogenize genetic variation, if extinction rate is sufficiently high compared to the effect of drift in local demes. We investigated these theoretical predictions empirically in social spiders that are highly inbred. Social spiders show intranest mating, female-biased sex ratio, and frequent extinction and colonization events, factors that deplete genetic diversity within nests and populations and limit gene flow. We characterized population genetic structure in *Stegodyphus sarasinorum*, a social spider distributed across the Indian subcontinent. Species-wide genetic diversity was estimated over approximately 2800 km from Sri Lanka to Himalayas, by sequencing 16 protein-coding nuclear loci. We found 13 SNPs in 6592 bp ( $\pi = 0.00045$ ) indicating low species-wide nucleotide diversity. Three genetic lineages were strongly differentiated; however, only one fixed difference among them suggests recent divergence. This is consistent with a scenario of metapopulation dynamics that homogenizes genetic diversity across the species' range. Ultimately, low standing genetic variation may hamper a species' ability to track environmental change and render social inbreeding spiders 'evolutionary dead-ends'.

**Introduction**

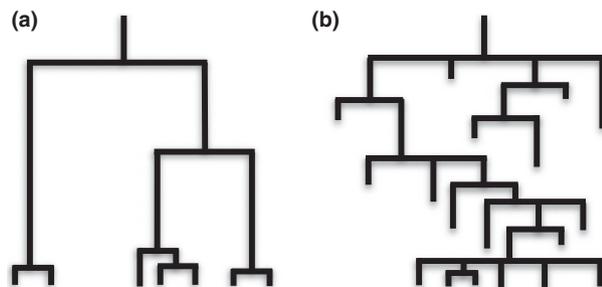
Theory predicts that inbreeding results in reduced effective population size ( $N_e$ ), which reinforces genetic drift and accelerates loss of genetic diversity, and may ultimately impair the potential of populations to respond to environmental challenges (Amos & Harwood, 1998; Charlesworth, 2003; Reed & Frankham, 2003). In the absence of gene flow, these processes are expected to partition genetic variation among populations and cause deep lineage differentiation (Charlesworth, 2003) (Fig. 1a). The divergence between lineages will, however, act to maintain species-wide genetic diversity. If

processes of local extinctions and colonizations are common, this may result in metapopulation structure with profound effects on genetic differentiation as gene flow may be enhanced to counteract divergence (Slatkin, 1987; Pannell & Charlesworth, 1999) (Fig. 1b). The extent to which metapopulation dynamics is expected to homogenize genetic variation across demes depends on migration rates among demes, extinction rates of demes, the effect of drift in the demes and whether recolonization occurs from a single or several extant populations (Wade & McCauley, 1988). Slatkin (1977) proposed a rule saying that even in the absence of gene flow genetic differentiation could be reduced if extinction rates are high enough relative to the effect of drift in the demes (Slatkin, 1977). The interaction between regular inbreeding and genetic homogenization would result in a reduction in genetic diversity

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both within and among demes, predicting overall low species genetic diversity. This scenario may have profound implications for evolutionary potential, but has received little empirical investigation.

Social spiders are permanently group living cooperative breeders that are characterized by lack of premating dispersal and strong inbreeding (Lubin & Bilde, 2007). Colonization occurs by fertilized females that disperse after mating, acting as propagules that establish new nests. Therefore, social spiders conform perfectly to the propagule-pool model of colonization, which assumes that available patches are colonized by members of a single extant population (Wade & McCauley, 1988). Social spiders of the genus *Stegodyphus* show a female-biased primary sex ratio of approximately 85% females, and reproduction is skewed towards less than half of the females in the nest (Lubin & Bilde, 2007). Similarly, most other social spider species show female-biased sex ratio (Lubin & Bilde, 2007). Female-biased sex ratio and reproductive skew are factors that rapidly deplete genetic variation within nests (Amos & Harwood, 1998). Genetic analyses indicate that individuals within nests can be almost completely homozygous (Lubin & Bilde, 2007), a nest can therefore be regarded as a breeding unit (Smith & Engel, 1994). Social spider populations consist of aggregations of nests (ecological populations), and both nests and populations experience high rates of turnover (Lubin & Crozier, 1985; Crouch & Lubin, 2001). Nest extinction rate was estimated higher than 20% per year. Frequent extinctions and establishment of nests are expected to quickly diminish genetic diversity within populations. High nest extinction rates will occasionally cause entire populations to go extinct (Crouch & Lubin, 2001). New populations are then founded by mated females from extant populations. These characteristics make social spiders an ideal study system to empirically test some of the theoretical predictions of the effect of metapopulation dynamics with propagule dispersal on genetic structuring and diversity of species (Wade & McCauley, 1988).



**Fig. 1** The expected evolutionary history in a scenario with no gene flow among lineages, which will cause deep divergences among lineages (a), and in a scenario with no gene flow, but strong metapopulation dynamics, which will cause low divergence among lineages (b).

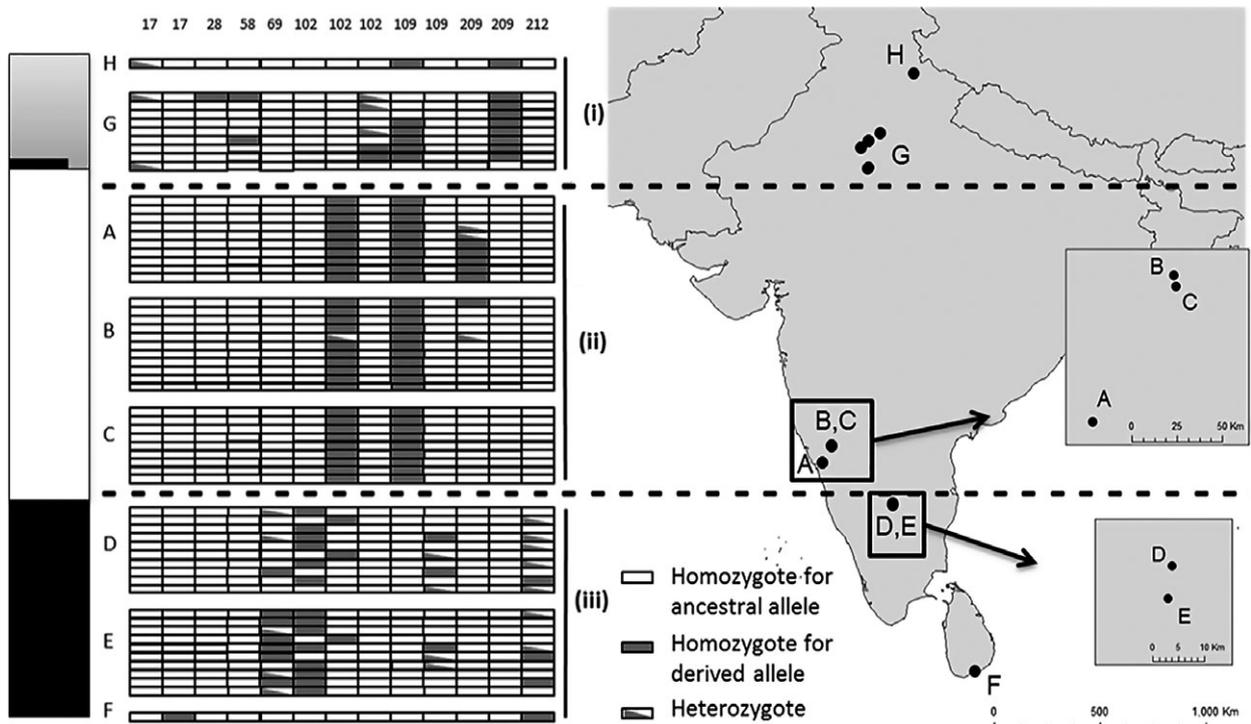
We investigated the level and structuring of genetic variation in the social spider *Stegodyphus sarasinorum* on the Indian subcontinent. Based on the mating system and the population dynamics characterizing social species, we predicted low genetic diversity within populations (Lubin & Bilde, 2007; Agnarsson *et al.*, 2013). Further, due to strong population turnover with propagule dispersal, we expect low species-wide genetic diversity and shallow divergence of genetic lineages, rather than deeply divergent lineages (Fig. 1).

## Materials and methods

### Sampling and sequencing

*Stegodyphus sarasinorum* Karsh (Eresidae) is one of three permanently social species in the *Stegodyphus* genus, and it is distributed in open and dry bush land in seasonal habitats across the Indian subcontinent and adjacent countries (Majer *et al.*, 2013; World Spider Catalog, 2014). A total of 61 individuals were sampled (one individual per nest). Empirical evidences suggest that individuals within nests can be almost completely homozygous (Lubin & Bilde, 2007) and a nest can be regarded as a breeding unit (Smith & Engel, 1994); therefore, we considered one individual representative of a colony. In the south, 10 individuals from each of five ecological populations were sampled (population A–E). A single individual was sampled in Sri Lanka (F). In the north, nine individuals were sampled from nests scattered over a large area of approximately 2000 square kilometres (G). A single individual was sampled in Himalaya (H) (Fig. 2, Table S1).

DNA was extracted using the DNeasy Blood & Tissue kit, Qiagen (Germantown, MD, USA). Sixteen protein-coding loci, chosen randomly from a list of 1345 alignments (Mattila *et al.*, 2012) including 16 exons and seven introns for a total of 6592 base pairs (of which 1752 bp intronic), were amplified in each individual (Table S2). Protein-coding loci were chosen to enable us to evaluate the adaptive potential of *S. sarasinorum*, under the assumption that a part of a species' adaptive potential is determined by the amount of standing genetic variation in protein-coding genes. Nuclear loci were chosen rather than the commonly used mitochondrial loci to avoid potential problems of mitochondria having a different evolutionary history than that of multiple nuclear loci (Ballard & Whitlock, 2004; Galtier *et al.*, 2009). Random loci were chosen to obtain unbiased diversity estimates. After amplification, the samples were stored in a  $-20^{\circ}\text{C}$  freezer until Sanger sequencing of the PCR products was performed. All chromatograms were inspected manually to check for heterozygous sites using BioEdit 7.0.8.0. (Hall, 1999) to secure that sequences of poor quality were excluded from the analysis.



**Fig. 2** Graphical representation of population structure, genetic diversity and geographical distribution of *S. sarasinorum*. The STRUCTURE output is shown on the left side of the figure, colours are associated with cluster affinity for each sample. The graph in the centre represents variable loci and their pattern of variation in each individual/cluster. Each line of the graph represents an individual and each column a variable position in a locus (names of the loci on top of the graph). Only variable loci have been included in the figure. i, ii, iii represent genetic lineages. The geographical distribution of the samples is shown in the map in the right side. The four GPS points present in the map for population H represent nine sampling localities (due to resolution is not possible to visualize all the sampling locations in this map).

## Data analyses

We used STRUCTURE 2.1 (Pritchard *et al.*, 2000) to delineate genetic clusters of the 61 individuals. In social spiders, nest propagation happens by single females already mated with their brothers within their natal nest resulting in very limited gene flow (Johannesen *et al.*, 2002, 2009). Therefore, we used the no-admixture model, which assumes that all of the genetic material from any given individual comes from one population rather than from mixed ancestry (Falush *et al.*, 2007). We used the no-admixture model and independent allele frequencies to determine the number of clusters,  $K$ , by comparing log-likelihood ratios produced by 10 iterations for  $K$  between 1 and 5. Each run consisted of 5 000 000 MCMC chain with a burn-in of 1 500 000 (30%). We implemented the Evanno *et al.* (2005) method to estimate the most likely value of  $K$ .

Haplotype data were generated prior to analyses using DNAsp 5 (Librado & Rozas, 2009). Species-wide genetic diversity ( $\pi$ ) was estimated for all samples, for each of three genetic lineages identified by

STRUCTURE, and within each of the five Southern populations (A–E).

DNAsp 5 (Librado & Rozas, 2009) was used to test for selection (Tajima's  $D$ ), recombination ( $R$ ) and to estimate diversity ( $\pi$ ). Before estimating the diversities, we corrected for sample size differences among genetic clusters identified by STRUCTURE 2.1 by down sampling the Southern genetic lineages (ii and iii), which contained more individuals than the genetic lineage from the North (i). Twenty random sequences from each lineage were drawn without replacement 20 times, and diversity estimates were obtained by averaging over the 20 resampled data sets.

Genetic differentiation ( $F_{st}$ ) was estimated by  $1 - \pi_s / \pi_t$ ,  $\pi_s$  being average  $\pi$  of the two clusters/populations under consideration and  $\pi_t$  the total  $\pi$  of the two clusters/populations under consideration.

## Results

None of the loci showed sign of selection (Tajima's  $D$ ) or recombination ( $R$ ).

Thirteen segregating sites were found across all loci (Fig. 2), nine in exons (one nonsynonymous, eight synonymous) and four in introns. Three of the segregating sites were singletons. Eight of the 16 loci sequenced were monomorphic. The STRUCTURE analyses showed the presence of three genetic clusters, representing distinct genetic lineages that also group geographically: (i) G and H; (ii) A, B, and C; and (iii) D, E and F (Fig. 2). One individual in population G was genetically more similar to lineage (iii) than to lineage (i) (result confirmed by resequencing).

Species-wide nucleotide diversity was estimated to  $\pi = 0.00045$  (Table 1). Diversities were estimated to  $\pi = 0.00029$ ,  $\pi = 0.00007$  and  $\pi = 0.00032$ , for genetic lineage i, ii and iii, respectively. For populations A–E, diversities were estimated to be  $\pi = 0.00005$  in A,  $\pi = 0.00004$  in B,  $\pi = 0$  in C,  $\pi = 0.00029$  in D and  $\pi = 0.00025$  in E.

The three genetic lineages were strongly differentiated with  $F_{st}$  values between 0.36 and 0.51.  $F_{st}$  among the genetic lineages was also estimated excluding the two individuals from Sri Lanka and Himalaya. The results of these analyses do not differ qualitatively from the reported  $F_{st}$  values above. Lineage ii and iii shared one polymorphism (locus 102) and had one fixed difference (locus 109, Fig. 2). Differentiation among populations A–E varied, but was generally low between populations from the same genetic lineage (ii:  $F_{st} = 0–0.42$ , iii:  $F_{st} = 0.06$ ). All five polymorphisms were shared between population D and E, whereas one polymorphism was shared between population A and B (Fig. 2).

## Discussion

Our analysis revealed 13 SNPs in 6592 base pairs ( $\pi = 0.00045$ ) and complete homozygosity of half of the loci sequenced, suggesting very low overall genetic diversity in *S. sarasinorum* compared to estimates from other arthropods, as there are no comparable data from

other spider species (Table 1). Population extinction and recolonization events are proposed to be prominent characteristics of social spiders (Crouch & Lubin, 2001; Lubin & Bilde, 2007), and metapopulation dynamics with propagule dispersal may under such circumstances result in decreased  $N_e$  and low species-wide genetic diversity (Wade & McCauley, 1988; Pannell & Charlesworth, 1999). Our finding of low diversity across the distribution range of *S. sarasinorum* is consistent with high population turnover and suggests homogenization over large geographical scale (Wade & McCauley, 1988). We note that the diversity estimate reported here may not reflect the true species-wide diversity of *S. sarasinorum*, because there may be genetic lineages we did not sample. Our estimate may therefore be an underestimate. However, the fact that the individuals from Sri Lanka and Himalaya were so genetically similar suggests that the hypothesized homogenization process can occur over large distances.

An important factor determining the structuring of a metapopulation is the number of migrants, that is gene flow, and their origin. Gene flow appears to be limited, if not absent, in our study species (Smith & Engel, 1994) and other social spider species (Lubin & Crozier, 1985; Avilés, 1992; Smith *et al.*, 2009), a factor that should promote strong divergence among lineages (Charlesworth & Willis, 2009). The highly separated genetic lineages grouping geographically conforms to expectations of limited gene flow, at least over the distances between sites investigated here. However, low overall diversity and low divergence between genetic lineages (only one fixed difference) indicate high turnover dynamics of local populations.

A recent study on *Daphnia magna* showed a very similar pattern. By comparing populations exposed and not exposed to high turnover dynamics, Walser & Haag (2012) showed that population turnover is responsible for low genetic diversity both in local populations and in the entire metapopulation and that it determines strong dif-

**Table 1** Diversity estimates ( $\pi$ ) of *S. sarasinorum* and a sample of available data for other arthropods and nematodes species with varying predicted population sizes based on distribution rates and mating systems. All  $\pi$  estimates are obtained from protein-coding loci. We note that substitution rates differ among species and loci and that estimates therefore are not 100% comparable.

Species	Average $\pi$	Geographical range	Mating system	Reference
<i>Stegodyphus sarasinorum</i>	0.00045	India and Sri Lanka	Sib mating	
<i>Drosophila sechellia</i>	Autosomal loci: 0.00065	Seychelles' archipelago		Legrand <i>et al.</i> (2009)
<i>D. melanogaster</i>	Autosomal loci: 0.00565*	Average over Europe and Africa		Andolfatto (2001)
<i>D. simulans</i>	Autosomal loci: 0.01163*	Average over Europe and Africa		
<i>Caenorhabditis elegans</i>	0.0004	Worldwide	Self-fertilizing hermaphrodites	Graustein <i>et al.</i> (2002)
<i>C. briggsae</i>	0.0043	Worldwide	Self-fertilizing hermaphrodites	
<i>C. remanei</i>	0.015	Worldwide	Cross-fertilizer	
<i>Daphnia arenata</i>	0.0013	USA	Cyclical parthenogen	Omilian & Lynch (2009)
<i>D. pulex</i>	0.0118	USA	Cyclical parthenogen	
<i>D. pulicaria</i>	0.0095	USA	Cyclical parthenogen	
<i>D. obtusa</i>	0.0052	USA	Cyclical parthenogen	

\**Drosophila melanogaster* and *D. simulans* estimates are reported as  $\theta_w$ , but  $\pi$  estimates were stated to be similar.

ferentiation among local populations (Walser & Haag, 2012).

Alternatively, recent demographic events such as a bottleneck or a founder event could explain the low diversity observed (Galtier *et al.*, 2000). Our data set does not provide enough resolution to detect such events due to too few segregating sites. However, there is evidence that the Indian climate has been relatively stable over the last decades (Sandel *et al.*, 2011), making this possibility less likely.

The loci sequenced in this study are all protein coding, which might cause selective constraints, so that the observed low diversity could be maintained by selection rather than by mating system and population dynamics. We found a slightly higher density of SNPs in introns compared to exons (1 per 438 bp vs. 1 per 537 bp). Also, in the recently sequenced genome of the social congener *S. mimosarum*, SNPs were denser in noncoding compared to coding sequence (1 per 2500 bp vs. 1 per 3500 bp) (Sanggaard *et al.*, 2014). These data suggest that although there is lower genetic variation in coding loci, this alone does not explain the low diversity estimate found here.

Our results suggest that genetic diversity can be homogenized over large areas despite no gene flow among populations, as predicted by strong population turnover (Slatkin, 1987; Wade & McCauley, 1988). Such dynamics leave the species with the challenge of adapting to changing environments with very little standing protein-coding genetic variation. This is part of the reason that social spiders have been hypothesized to be 'evolutionary dead-ends' (Agnarsson *et al.*, 2006), characterized by low diversification and increased risk of extinction (Charlesworth, 2003). Our findings further begs the question of how the species manages to occupy a wide niche with very different climates as such as those of Himalaya and Sri Lanka within their species range.

## Data accessibility

Details supporting this article have been uploaded as Supplementary Information. DNA sequences: GenBank accessions numbers: KM880193–KM881107.

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### Supporting information

Additional Supporting Information may be found in the online version of this article:

**Table S1** Approximate distances between populations based on GPS points.

**Table S2** Information on primer pairs used.

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