

The age and evolution of sociality in *Stegodyphus* spiders: a molecular phylogenetic perspective

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Social, cooperative breeding behaviour is rare in spiders and generally characterized by inbreeding, skewed sex ratios and high rates of colony turnover, processes that when combined may reduce genetic variation and lower individual fitness quickly. On these grounds, social spider species have been suggested to be unstable in evolutionary time, and hence sociality a rare phenomenon in spiders. Based on a partial molecular phylogeny of the genus *Stegodyphus*, we address the hypothesis that social spiders in this genus are evolutionary transient. We estimate the age of the three social species, test whether they represent an ancestral or derived state and assess diversification relative to subsocial congeners. Intraspecific sequence divergence was high in all of the social species, lending no support for the idea that they are young, transient species. The age of the social lineages, constant lineage branching and the likelihood that social species are independently derived suggest that either the social species are ‘caught in sociality’ or they have evolved into cryptic species.

Keywords: evolvability; evolutionary dead end; inbred lineages; social spider; stasis

1. INTRODUCTION

Cooperative breeding or sociality is rare in spiders. Only about 20–30 species are considered social (non-territorial permanently social *sensu* Avilés 1997). Nevertheless, sociality has evolved independently in several families of spiders (Kullmann 1972; Avilés 1997). Social spiders live in colonies with high levels of inbreeding and high relatedness among colony members (Lubin & Crozier 1985; Roeloffs & Riechert 1988; Smith & Engel 1994; Smith & Hagen 1996; Johannesen *et al.* 2002). This mating system, combined with high turnover rates of colonies (Smith & Hagen 1996; Crouch & Lubin 2001) and both dispersal and colony founding by mated females, prevents gene flow and provides conditions that may purge genetic variation and lower individual fitness quickly.

The inbred nature of social spiders led Wickler & Seibt (1993) to speculate that social species are unstable in evolutionary time and constitute evolutionary dead ends (Futuyma & Moreno 1988), which could explain the rarity of social spiders. Two features might limit the ability to diversify and speciate, namely extreme inbreeding and obligate group living, both of which narrow the species’ ecological niche. Furthermore, the high turnover of colonies may reduce the potential for independently

evolving colonies to proliferate until speciation (Avilés 1997). Alternatively, the independent nature of colony lineages could enhance speciation in some circumstances, especially if the colony-lineage extinction rate is low and the detrimental effects of inbreeding can be overcome (Smith 1986). Johannesen *et al.* (2002) recently showed divergent mtDNA lineages in social *Stegodyphus dumicola*. This finding indicates that *S. dumicola* is unlikely to be a young transient species, although they did not provide evidence for speciation as such.

We use the genus *Stegodyphus* (Eresidae) to test the hypothesis that social spiders are evolutionary transient ‘dead end’ species and have not undergone diversifying speciation. Based on a partial molecular phylogeny and sequence divergence, we tested (i) the age, (ii) ancestral or derived state, and (iii) species diversification (or evolvability) of social *Stegodyphus* spiders. The genus consists of 15–17 species and is divided into three species groups. Each species group includes one social species and several subsocial species with extended maternal care (Kraus & Kraus 1988). In this study, we analyse eight *Stegodyphus* species, including the three social species and their respective subsocial sister species (*sensu* Kraus & Kraus 1988).

The degree of diversification of *Stegodyphus* social spiders was inferred from a partial phylogeny of the genus and intraspecific divergence from geographical samples of the social species. First, we tested whether the social species belong to different species groups as suggested by Kraus & Kraus (1988). If social species are young and derived, we

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Table 1. List of *Stegodyphus* species and social species groups with distribution ranges, as described by Kraus & Kraus (1988). (Both sexes (m, male; f, female) are described from all except three species. Species included in our analysis are shown in bold.)

species group	species	sex	social status	distribution range	country of origin	GenBank <i>COI</i>	accession <i>16S</i>	numbers <i>ND1</i>
<i>S. dufouri</i>	<i>sarasinorum</i>	mf	social	India–Sri Lanka	India (1994) (2004)	DQ973163 DQ973164	DQ973144 DQ973145	DQ973128 DQ973129
	<i>pacificus</i> ^{a,b}	mf	subsocial	India–Iran	—	—	—	—
	<i>dufourii</i> ^{a,b}	mf	subsocial	Northern Africa	Egypt	DQ973159	DQ973142	DQ973127
	<i>bicolor</i>	mf	subsocial	Namibia	Namibia	DQ973155	DQ973137	DQ973126
	<i>manicatus</i>	mf	subsocial	West Africa, North East Africa	—	—	—	—
<i>S. mirandus</i>	<i>dumicola</i> ^c	mf	social	southern Africa	Namibia (B2) (A5)	DQ973156 DQ973157	DQ973138 DQ973139	AF494438 AF494435
	<i>tentoriicola</i> ^{a,c}	mf	subsocial	South Africa	South Africa	DQ973158	DQ973142	AF494440
	<i>mirandus</i>	mf	subsocial	Eastern India	—	—	—	—
	<i>tibialis</i>	mf	subsocial	Southern India	—	—	—	—
	<i>nathistmus</i>	mf	subsocial	Morocco	—	—	—	—
	<i>lineatus</i>	mf	subsocial	Mediterranean Kazakhstan	Israel–Judea Israel–Negev	AY611798 AY611804	DQ973140 DQ973141	AY611785 AY611780
<i>S. africanus</i>	<i>mimosarum</i>	mf	social	Southern and Eastern Africa	South Africa	DQ973160	DQ973146	DQ973130
	<i>africanus</i> ^a	mf	subsocial	Southern Africa	Madagascar South Africa	DQ973161 DQ973162	DQ973147 DQ973148	DQ973131 DQ973132
	<i>hildebrandti</i>	mf	subsocial	East Africa	—	—	—	—
	<i>sabulosus</i>	f	subsocial	Southern and Eastern Africa	—	—	—	—
	<i>lineifrons</i>	f	subsocial	East Africa	—	—	—	—
	<i>tingelin</i> ^d	m	subsocial	Cameroon	—	—	—	—

^a Nearest sister species to the respective social species (Kraus & Kraus 1988).

^b Distinguishable only by leg I/promosa ratio, indistinguishable by genitalia; considered by Kraus & Kraus (1988, 1990) as geographical variants of the same species.

^c Morphologically very similar, particularly females.

^d Only one male described.

expect them to occur in shallow phylogenetic lineages relative to the age of the genus as a whole and to be tip clades. If the social species evolved independently, they should not be monophyletic. If the social species are monophyletic, they must have evolved from a common ancestor. In this case, they were able to undergo diversifying speciation despite the difficulties imposed by severe inbreeding and ecological constraints. In the alternative possibility that they are phylogenetically isolated and old tip clades, diversification may be constrained. However, an estimate of species diversification depends on the speciation rate being greater than the extinction rate. New social species may evolve from parent social species, but they become extinct at a rate that limits the proliferation of new social species. In this case, social species might be unstable in evolutionary time and the potential of social lineages to speciate is underestimated. The age of the social species provides an indirect assessment of this possibility. If social species have distinct within-species lineages and if these are old, then one may conclude that social species are stable in evolutionary time. This would suggest that social species are missing because they never evolved, rather than because they became extinct.

2. MATERIAL AND METHODS

(a) Species

Table 1 shows species and putative species groups of *Stegodyphus*, modified from Kraus & Kraus (1988). The division into species groups was defined principally by

different character expressions in the male and female genitalia, but a quantitative phylogenetic analysis was not performed (Kraus & Kraus 1988). In the present study, we had access to eight *Stegodyphus* species, including the three social species and their respective sister species. The social species, *Stegodyphus mimosarum* and *S. dumicola*, were accompanied by *Stegodyphus africanus* and *Stegodyphus tentoriicola*, respectively. For the third social species, *Stegodyphus sarasinorum*, we included the sister species *Stegodyphus dufouri*. Kraus & Kraus (1988) originally classified the Indian *Stegodyphus pacificus* as *S. sarasinorum*'s sister species, but noted that *S. pacificus* is morphologically indistinguishable from *S. dufouri*. Later, they considered *S. dufouri* and *S. pacificus* as geographical variants (Kraus & Kraus 1990). Two additional subsocial species, *Stegodyphus lineatus* and *Stegodyphus bicolor*, belong to the clades that include two social species *S. dumicola* and *S. mimosarum*, respectively (*sensu* Kraus & Kraus 1988).

An estimate of age was gained from intraspecific sequence divergence. *Stegodyphus mimosarum* and *S. dumicola* and the subsocial *S. lineatus* were analysed from populations that were geographically dispersed. The *S. dumicola* and *S. lineatus* sequences included were based on the most divergent lineages from previous studies (Johannesen *et al.* 2002, 2005). The intraspecific sequence divergence in *S. sarasinorum* was based on two samples collected around Bangalore, India in 1990 (Smith & Engel 1994) and 2004. To confirm individual specificity, we initially sequenced two to three individuals at the *16S* and NADH dehydrogenase subunit1 (*ND1*) locus in

all species and their geographical samples except *S. africanus*, where the cytochrome oxidase subunit 1 (*COI*) locus was tested (see below). As these intraspecific sequences were identical or differed by not more than 4 bp (*COI* in *S. africanus*, 0.4% divergence), we limited phylogenetic analyses to one individual of each taxon. Owing to a number of *Stegodyphus* species for which molecular data are missing, we do not attempt to establish a phylogeny of the entire genus.

(b) DNA procedure

All individuals were analysed for a double-stranded mtDNA template (1945 characters including gaps), consisting of a partial *ND1* (361 bp), 16S ribosomal RNA (*16S*) and tRNA (616 characters), *COI* (947 bp). *ND1* was amplified via PCR using the primers LR-N-12945: 5'-CGA-CCT-CGA-TGT-TGA-ATT-AA-3' and N1-J-12261: 5'-TCG-TAA-GAA-ATT-ATT-TGA-GC-3' (Hedin 1997); *16S* primers LR-J-12887: 5'-CCG-GTT-TGA-ACT-CAA-ATC-ATG-T-3' and LR-N-13398: 5'-CGC-CTG-TTT-AAC-AAA-AAC-AT-3' (Simon *et al.* 1994); *COI* primers C1-J-1751: 5'-GAG-CTC-CTG-ATA-TAG-CTT-TTC-C-3' and C1-N-2776: 5'-GGA-TAA-TCA-GAA-TAT-CGT-CGA-GG-3' (Hedin & Maddison 2001). Amplification conditions were identical for all primer pairs and are given in Johannesen & Veith (2001). The *ND1* amplification product consists of about 180 bp *16S* bordering the *ND1* sequence. Hence, the combined *16S* sequence is a product of the *16S* sequence and 180 bp amplified by the *ND1* primers.

PCR products were sequenced using an ABI-377 automatic sequencer. Sequences were aligned using the programme SEQUENCE NAVIGATOR (ABI). Initially, each sequence was aligned with a reference sequence and all mutations were checked. Having confirmed each mutation, all sequences were aligned with CLUSTAL in SEQUENCE NAVIGATOR (ABI; default settings).

(c) Data analysis

We tested for neutral nucleotide evolution of the protein-coding regions (*ND1* and *COI*) using D^* (the number of unique mutations among all sequences relative to the total number of mutations) and F^* (the average number of nucleotide differences between pairs of sequences) tests of Fu & Li (1993), as implemented in DNASP v. 4.0 (Rozas *et al.* 2003). *Post hoc* tests for non-neutral evolution of the coding *COI* and *ND1* genes were performed with the MacDonal–Kreitman test using DNASP v. 4.0.

Phylogenetic trees were estimated for all the three genes separately and for the combined sequence. Phylogenetic analyses were performed applying maximum parsimony (MP), distance analysis with sequences joined by neighbour joining (NJ) and maximum likelihood (ML) with PAUP v. 4.08b for the Macintosh (Swofford 1999) and with Bayesian inference (MRBAYES v. 3.0B4; Huelsenbeck & Ronquist 2001). In MP, all the characters were weighted equally. Indels were excluded from the analysis. Haplotype relationships were analysed with a heuristic search with random addition of sequences. For the NJ analysis, we chose the distance model with the highest likelihood ratio found by MODELTEST v. 3.7 (Posada & Crandall 1998). The likelihood ratio was estimated among in-group haplotypes (eresid species). The gamma distribution was estimated from the data. Fifty per cent majority-rule consensus trees based on bootstrap search were computed for all tree algorithms. MP and NJ were performed with 2000 replicates. Owing to

computational limits, ML was performed with only 200 resamplings. Bayesian inference was conducted with 1 000 000 generations, with an initial burn-in of 1000 trees. All simulations were defined to consider coding and/or non-coding sequences. Each sequence analysis was performed three times to verify that we had found the optimal likelihood level.

The phylogeny was rooted using six spider species: *Eresus cinnaberinus* (GenBank accession numbers: *ND1* and *16S*, AF374171; *COI*, DQ973153); *Eresus walckenaeri* (*ND1* and *16S*, AF374181; *COI*, DQ973154) and *Gandananemo spenceri* (DQ973133, DQ973149, DQ973165) were used as genus comparisons within the family Eresidae; *Uroctea durandi* (Oecobiidae) as Eresoidea comparison (DQ973124, DQ973150, DQ973166); and *Meta menardi* (Metidae; DQ973135, DQ973151, DQ973167) and *Cyrtophora citricola* (Araneidae; DQ973136, DQ97312652, DQ973168) were used for out-group rooting. *Uroctea durandi*, *M. menardi* and *C. citricola* were highly but equally divergent to *Stegodyphus*. To control for false rooting owing to long-branch bias, all phylogenetic analyses were repeated with *G. spenceri* as out-group and *Stegodyphus* and *Eresus* as in-groups. GenBank accession numbers of *Stegodyphus* are listed in table 1.

We used two approaches to gain insight into the diversification process. First, we inferred gene saturation. If a phylogenetic topology is unresolved at the base of the tree and the gene is not saturated, this may indicate rapid lineage divergence. Gene saturation was inferred by comparing uncorrected pairwise distances of each of the three genes to their respective branch lengths in MP trees. This method is based on the assumption that the actual amount of evolutionary change should be at least the same as the minimum estimates of change inferred by parsimony (Page & Holmes 1998). Second, we tested the null hypothesis that the phylogeny of *Stegodyphus* evolved as a result of a constant-rates birth–death process (Pybus & Harvey 2000), with the programme GAMMASTATISTIC (Griebeler 2004). The constant-rates model is rejected if the internal nodes of a reconstructed phylogeny are closer to the root than expected under a pure birth model. The sign of the gamma statistic is an indication of the relationship between the birth and death process: if $\gamma > 0$, a phylogeny's internal nodes are closer to its tips than expected under a pure birth model (i.e. it has a high relative death rate), whereas if $\gamma < 0$, the internal nodes are closer to the root than expected under a pure birth model. For the constant-rates birth–death test, we estimated internode distances from a UPGMA phylogram calculated with PHYLIP (Felsenstein 2002). The UPGMA phylogram was calculated from the total sequence (1947 characters) with the distance model estimated by MODELTEST v. 3.7.

3. RESULTS

Gene sequence and phylogeny statistics are summarized in table 2 of the electronic supplementary material. Phylogenetic analyses were performed for the three genes *ND1*, *COI* and *16S*, both separately and combined. All heuristic searches ML, NJ and MP of the total sequence found the most likely/parsimonious tree with identical topologies: MP, tree length 2591, CI=0.535, RI=0.522; NJ distance search, ME score=2.91367; ML most likely tree, $-\ln L=13308.983$. The topology was identical to that found by Bayesian search. Figure 1 shows the ML tree calculated by a heuristic search from the total sequence

Figure 2 of the electronic supplementary material shows gene saturation of *Stegodyphus* and *Eresus* sequences. *ND1* reaches a plateau and is influenced by gene saturation in deep time, whereas *16S* is only slightly affected by gene saturation. *CO1* shows an intermediate pattern by approaching saturation, but then appears to evolve steadily. This suggests that gene saturation cannot alone explain the unresolved basal radiation.

The phylogenetic branching pattern for all *Stegodyphus* and *Eresus* sequences did not differ significantly from the constant-rates birth–death process, $\gamma = -1.137$, $p > 0.05$.

4. DISCUSSION

The data presented here do not support the hypothesis that social *Stegodyphus* spiders are transient in evolutionary time. High sequence divergence within all three social species suggests that they are old species. Sequence divergence of *ND1* within *S. dumicola* and *S. mimosarum* was 5 and 7%, respectively. This corresponds to one-quarter to one-third of the total sequence divergence among species groups! This level of sequence divergence is double that between two phylogeographical lineages of the subsocial *S. lineatus*. The partition into species groups (*sensu* Kraus & Kraus 1988) was confirmed except for *S. lineatus*, which was divergent enough to belong to a fourth ‘group’ (clade). The heuristic searches suggested that *Stegodyphus* might be paraphyletic, but the radiation of the four major *Stegodyphus* clades was not resolved with ML, MP or NJ bootstrap analyses. Concerning the possible paraphyletic state of *Stegodyphus*, we can only note that it is puzzling and needs more research. So far, no formal morphological phylogenetic analysis has investigated the relationship among the eresid genera.

It is difficult to evaluate the minimum age of the social species. If one considers that intraspecific gene lineages are monophyletic, i.e. gene trees correlate with species trees in all social species, and coalescence patterns from geographical samples of *S. dumicola* and *S. mimosarum* converge on a common social ancestor, respectively (Johannesen *et al.* 2002; J. Johannesen & R. Moritz 2006, unpublished data), a social species is probably at least as old as the intraspecific sequence divergence. The calibration of the divergence rates poses two problems. First, the relative rates of evolution of *ND1* and *CO1* observed here do not allow a comparison with the literature. Rates of *ND1* and *CO1* evolution are given traditionally as *ca* 2 and 2.3% per million year, respectively (Desalle *et al.* 1987; Brower 1994; but see Pruser & Mossakowski 1998), resulting in a *ND1/CO1* ratio of about 1. The eresid spiders deviated significantly from this value, with *ND1* evolving 1.5 times faster than *CO1*. Second, our study’s only geological calibration point (South Africa and Madagascar) for sequence divergence may give misleading estimates of age. Several studies have found conflict between the fossil record or molecular divergence and the presumed Early Cretaceous origin of the endemic fauna (Vences *et al.* 2001 and references therein). Estimating age from sequence data may also be influenced by taxa-specific evolutionary rates, e.g. high sequence divergence within the social species may be a consequence of sociality itself. However, evidence suggests that this is not the case. First, we did not observe

increased non-synonymous substitution rates in social species, i.e. there was no evidence for selection at the two loci. Second, sequence divergence among allopatric populations of the eresids *S. lineatus* and *E. cinnaberinus* show similar high divergence as well as similar constant branching patterns (Johannesen & Veith 2001; Johannesen *et al.* 2005), and divergence at this level has been found in other spiders with restricted niches (Hedin 1997; Bond *et al.* 2001; Hedin & Wood 2002). Third, although *CO1* and *ND1* are linked on the mitochondrial genome, the likelihood that two coding genes simultaneously increase mutation rates seems small. Despite these difficulties and the uncertainty regarding the exact age, it is evident that intraspecific divergence was very high. Even an elevated rate of 3% per million year for *ND1* would set the diversification at *ca* 2 Myr between intraspecific *S. dumicola* and *S. mimosarum* lineages and at *ca* 1 Myr between *S. sarasinosum* lineages. In this context, it is not important whether the age is 1, 2 or 3 Myr; rather, it is important that social spiders as species may bridge very long periods of time. This level of divergence is incompatible with the idea that social *Stegodyphus* spiders represent a transient state and are evolutionarily unstable.

If social species are old and derived, it may indicate that they are unable to evolve cladogenetically. Three lines of evidence suggest that the social *Stegodyphus* are derived and that sociality evolved independently in the three groups. First, rather than being monophyletic, the social species occur in old, separate species groups. This indicates that they were not derived from a single common ancestor (but see below). Second, intraspecific divergence was large, but still less than the divergence from their respective sister species (*sensu* Kraus & Kraus 1988). Lastly, *S. sarasinorum* lineages were tip clades within its group consisting of three analysed species. Ideally, at least two subsocial species are needed for an estimate of the derived state of social species within groups. We could only test the within-group derived state for this one group, because *S. lineatus*, the third species in the *S. dumicola* group (according to Kraus & Kraus 1988), clustered into a separate, fourth species group. Although the most parsimonious explanation of the data suggests a derived state for each social species, this result should be considered as preliminary because not all *Stegodyphus* species were included in the analysis. Caution should also be taken because the possible paraphyletic state of *Stegodyphus* means that we cannot entirely dismiss the possibility, however unlikely, of an ancestral state of sociality.

The ability to evolve may be defined in the short term as adaptation to changing environments or in the longer term as the ability for cladogenetic speciation. In the former context, the presence of old social lineages in *Stegodyphus* shows survival over evolutionary time, which would require tracking changing environments. Whether species diversification occurred in social lineages is less clear. Constancy in the rate of lineage diversification shows that social and subsocial sequences (i.e. lineages) do not differ in the ability to diversify genetically; constant diversification is also found in geographical samples of *S. dumicola* (Johannesen *et al.* 2002) and *S. mimosarum* (J. Johannesen & R. Moritz 2006, unpublished data). The birth–death model gamma statistics were negative for all sequences, although not

statistically significant, suggesting that internal nodes are closer to the root than expected. This and the ancient splits between social and subsocial species together imply that following an initial rapid radiation cladogenesis at the genus level, in general, may be slow.

Similar divergence patterns in all social *Stegodyphus* species suggest the existence of similar life-history processes that, on the one hand, are able to sustain sociality through time, but, on the other hand, limit cladogenesis. It could be that social species are 'caught in sociality' with limited ecological-morphological potential for change. The question arises whether the divergent intraspecific lineages belong to cryptic species. Madagascan *S. mimosarum*, for example, differ slightly from South African specimens and were described by Simon (1906, cited in Kraus & Kraus 1988) as *Stegodyphus simplicifrons* (females) and *Stegodyphus corallipes* (males). Our data support an ancient genetic lineage of Madagascan *S. mimosarum*, and it may provide evidence for the existence of two distinct species with allopatric distributions.

If social *Stegodyphus* can be stable in evolutionary time, why then are so few spiders social in general? Is it the difficulty of jumping the barrier to sociality rather than maintaining it once acquired (Bilde *et al.* 2005)? Are social species constrained so that the intraspecific lineages do not diversify unless they revert to subsociality? In a discussion of why there are species, Coyne & Orr (2004) pointed out that it is relatively easy to delimit species-clusters (indicating cladogenesis) in obligatory sexual and asexual species, but less so in species with intermediate sexual reproduction systems. Social spiders in a sense have an 'intermediate' reproductive system. They are highly inbred (Lubin & Crozier 1985; Roeloffs & Riechert 1988; Smith & Engel 1994; Smith & Hagen 1996; Johannesen *et al.* 2002), probably experiencing only sporadic outbreeding (T. Bilde 2004, unpublished data). If gene flow between lineages is rare, social spiders might be considered 'clonal'. Hence, a tentative explanation for the paucity of social species in spiders may be that niche space is not available for them to diversify ecologically while occasional gene flow between colonies prevents colonies from evolving independently as quasi species. This does not explain why intraspecific social spider lineages are ancient, but it may suggest why species are difficult to characterize. Niche space may be highly important for spiders to evolve morphologically. A decoupling of ecological specialization and morphological change from lineage divergence seems common in spiders with narrow ecological niches (Hedin 1997; Bond *et al.* 2001; Johannesen & Veith 2001; Hedin & Wood 2002).

In summary, we show that in *Stegodyphus*, sociality is old and social species are probably independently derived, and lineage divergence is constant but cladogenesis is constrained (as far as morphotypes are concerned). It is unclear whether diversification and cladogenesis are constrained at the genus level or solely at the social species level. Comparative studies of social and subsocial spiders in other families are needed to test the generality of these relationships.

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