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Evolutionary transitions towards eusociality in snapping shrimps

Solomon Tin Chi Chak[1,2]*, J. Emmett Duffy[1†], Kristin M. Hultgren[3] and Dustin R. Rubenstein[2]

Animal social organization varies from complex societies where reproduction is dominated by a single individual (eusociality) to those where reproduction is more evenly distributed among group members (communal breeding). Yet, how simple groups transition evolutionarily to more complex societies remains unclear. Competing hypotheses suggest that eusociality and communal breeding are alternative evolutionary endpoints, or that communal breeding is an intermediate stage in the transition towards eusociality. We tested these alternative hypotheses in sponge-dwelling shrimps, *Synalpheus* spp. Although species varied continuously in reproductive skew, they clustered into pair-forming, communal and eusocial categories based on several demographic traits. Evolutionary transition models suggested that eusocial and communal species are discrete evolutionary endpoints that evolved independently from pair-forming ancestors along alternative paths. This 'family-centred' origin of eusociality parallels observations in insects and vertebrates, reinforcing the role of kin selection in the evolution of eusociality and suggesting a general model of animal social evolution.

Although sociality in the form of cooperative group living[1] is widespread among animals, the structure of animal societies varies widely[2–6]. While many species associate temporarily or permanently in the minimal social group consisting of a mated pair, many species form larger groups in which the partitioning of reproduction (that is, reproductive skew[7]) ranges from low to highly skewed[8]. Since reproductive skew is a trait of the social group that varies continuously among species[8], it has been used to distinguish among and define different forms of social organization in animals as taxonomically diverse as insects and vertebrates[8–11]. At one extreme are the eusocial species, which are defined by overlapping generations, cooperative care of young, and a reproductive division of labour where many group members are temporarily or permanently sterile. In these species, skew is typically high because one or a few individuals monopolize the groups' reproduction[12]. In contrast, skew is generally lower in more egalitarian societies where reproduction is shared among many, or even all, group members[3,4,13]. Given that reproductive skew is only one of many interconnected traits that characterize social syndromes, determining whether these different forms of social organization—high- versus low-skew societies—are variations on a common theme[9] or represent independent and alternative social syndromes[10,13] remains a fundamental problem in biology that requires testing alternative models of social evolution.

Two alternative models of social evolution have been proposed to explain the evolutionary transition to eusociality from a non-social ancestor. In insects, it is generally accepted that eusociality arose through what has become known as the 'subsocial model'. This model suggests that eusocial species evolved from an intermediate ancestor whose immature offspring remained in the parental nest under the mother's care, forming an aggregation of related individuals that did not interbreed[14,15]. The subsocial model proposed for insects is similar to the 'extended-family model' proposed for vertebrates, in which high-skew societies evolved through the retention of offspring[16]. These ideas are further elaborated in the 'lifetime monogamy hypothesis'[15,17], which suggests that high relatedness among parents and offspring is a necessary precondition for eusociality to evolve. An alternative to these family-centred models of social evolution is the 'parasocial model'[14], which suggests that eusocial insects evolved from an intermediate ancestor where unrelated breeders of the same generation aggregated to form 'communal groups' characterized by low skew. Recent synthesis suggests that communal breeding societies with low skew and low genetic relatedness among group members are more common than once thought[1] in both insects (where females share a domicile but do not cooperatively care for young[18]) and vertebrates (where females share a domicile but do cooperatively care for young[1,3,4]). In essence, these alternative hypotheses (the subsocial and parasocial models) for the evolution of extreme reproductive altruism disagree on whether or not eusocial societies passed through a low-skew, intermediate form of social organization (such as communal breeding) during their evolution. Moreover, these models also disagree about whether high relatedness among the initial group members was a key prerequisite for advanced sociality or a subsequent consequence of group-living, a question that has generated intense debate[19,20]. Therefore, testing whether communally breeding species represent an intermediate stage in the evolution of eusociality remains crucial to understanding the evolution of animal sociality.

Clarifying the evolutionary transition to eusociality may also help resolve a long-standing debate in social evolution—whether social organization should be viewed as a continuous or discrete trait. The concept of a eusocial continuum treats eusocial species as an endpoint along a continuum of reproductive options, which implicitly assumes that eusocial species with high reproductive skew evolved via low-skew intermediates (that is, via the parasocial hypothesis). In contrast, the alternative idea that eusociality is a qualitatively and evolutionarily distinct form of social organization[8,9,21] marked by unique adaptations such as the lack of reproductive

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[1]Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, Virginia 23062, USA. [2]Department of Ecology, Evolution and Environmental Biology, Columbia University, New York, New York 10027, USA. [3]Biology Department, Seattle University, Seattle, Washington 98122, USA. [†]Present address: Tennenbaum Marine Observatories Network, Smithsonian Institution, PO Box 37012 NHB MRC 106, Washington DC 20013, USA. *e-mail: tc2856@columbia.edu

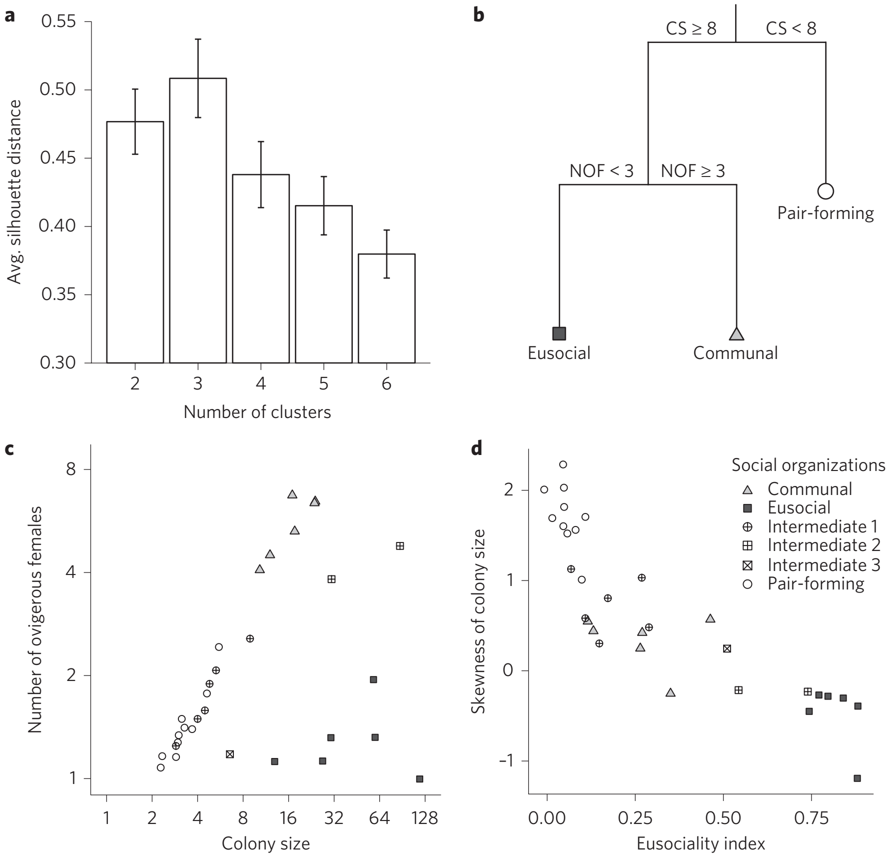


Figure 1 | Demographic classification of social states in *Synalpheus* shrimp. **a**, Optimum number of clusters (social categories) as determined from average silhouette distances (s_i) from PAM analyses with different k (number of clusters) and different input variables. The high s_i for $k = 3$ indicates that *Synalpheus* species are best separated into three clusters. Error bars indicate standard errors ($n = 7$). **b**, Classification tree used to characterize the three clusters, namely pair-forming, communal and eusocial, by colony size (CS) and the number of ovigerous females (NOF). **c**,**d**, Bivariate plots of two sets of variables (number of ovigerous females and colony size, and skewness of colony size and eusociality index) that resulted in the highest average s_i ($n = 31$). Pair-forming, communal and eusocial species were unequivocally identified in all PAM analyses regardless of input variables. Intermediate 1 were clustered with either pair-forming or communal species. Intermediate 2 was clustered with either communal or eusocial species. Intermediate 3 was clustered with either pair-forming, communal or eusocial species. In **c**, the number of ovigerous females and colony size are shown on geometric scales.

totipotency[15] or behaviourally distinct castes[22] is consistent with the subsocial hypothesis in which low-skew societies evolved along their own evolutionary path, and are not intermediate social forms on the path towards eusociality. However, whether societies characterized by different forms of social organization evolved continuously along an axis of reproductive skew or along divergent evolutionary paths towards different states of social organizations (for example, models 1 versus 2 in Results) has yet to be tested broadly outside of the Hymenoptera[23,24].

Examining the evolutionary transition to eusociality is difficult in most insects because eusociality appears to be ancient and ancestral to many lineages (such as in ants[25], termites[26], corbiculate bees[27] and allodapine bees[28]), but is sometimes incipient or even lost all together[27,29]. Moreover, empirical tests of the subsocial versus parasocial models in the Hymenoptera have produced mixed results. For example, the subsocial model was supported by comparative analysis in the halictid bees[23] but not the vespid wasps[24]. The strongest evidence in support of the subsocial model is the fact that all advanced eusocial insects evolved from species that were monogamous throughout their lifetimes[15,30], with offspring that remain in their natal nest being full-sibs. Thus, although it is generally accepted that eusociality in insects is likely to have evolved via the subsocial model, incomplete empirical evidence for this hypothesis[23,24] means that we still lack a complete understanding of how eusocial societies evolved, especially outside of the insects.

The snapping shrimp genus *Synalpheus* provides an opportunity to independently study the evolutionary transition towards eusociality because these marine animals not only exhibit nearly the full range of forms of animal social organization, but have also undergone at least four relatively recent independent origins of eusociality[11]. The ~45 West Atlantic species in the gambarelloides species group of *Synalpheus*[31] live obligatorily within the canals of sponges[32] and vary in social organization from pair-forming to communal breeding to eusociality[31]. Pair-forming is the ancestral state shared by most other alpheid snapping shrimps[33]. Pair-forming *Synalpheus* species live in sponges that typically contain only a single breeding pair[31]. Communal species live in groups with multiple breeding pairs and roughly equal ratios of adult males and females[34]. Eusocial species typically have a single 'queen' or at most a few queens, and up to several hundred non-breeding individuals that defend the sponge[35] and retain the ability to reproduce[36]. Data on within-colony relatedness suggests that colonies of eusocial *Synalpheus* species form through the accumulation of offspring[11,35], whereas communal species may form through the aggregation of unrelated pairs[31]. Because of its social diversity and the multiple independent origins of eusociality, *Synalpheus* offers an opportunity to test the alternative models of subsocial versus parasocial evolution of eusociality.

Here we used data from a collection of *Synalpheus* snapping shrimps amassed over nearly 30 years to understand the evolutionary transition from pair-forming groups towards eusociality.

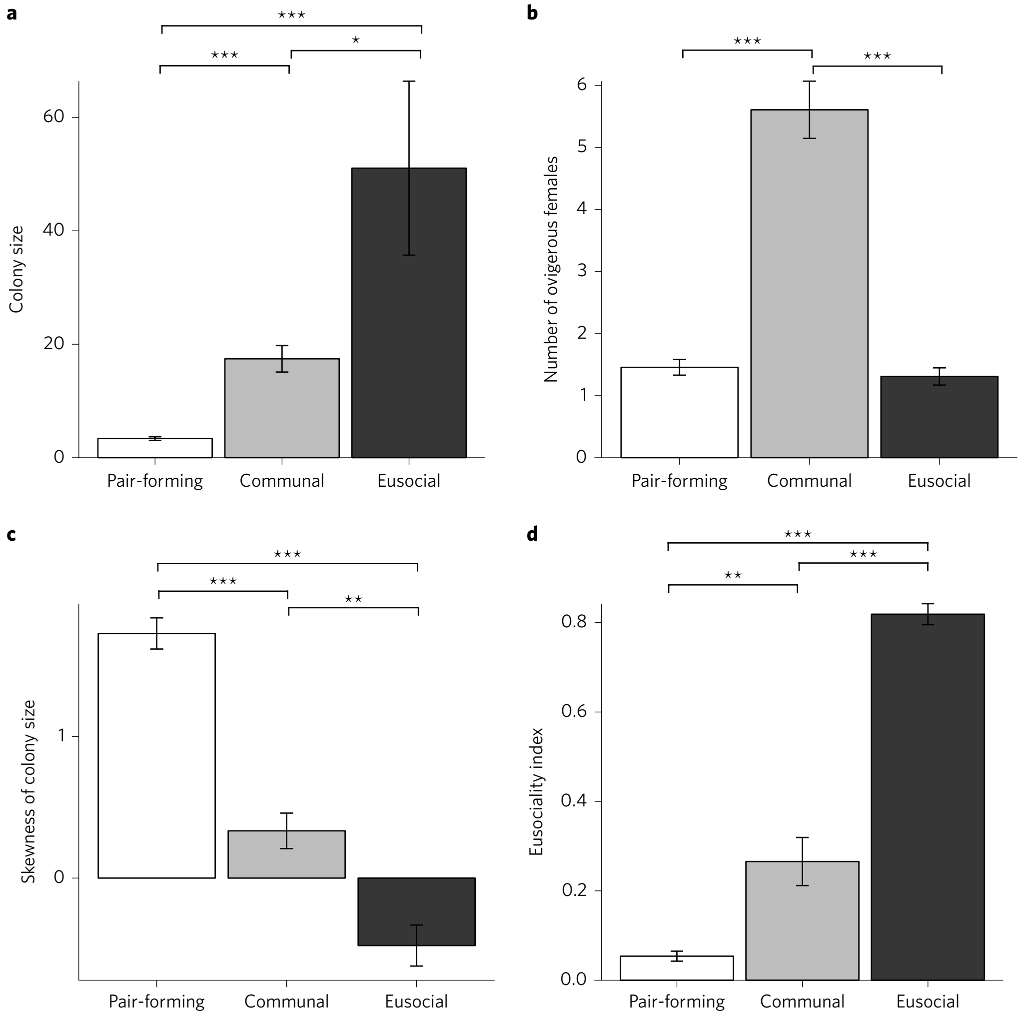


Figure 2 | Demographic properties of pair-forming, communal and eusocial species. a–d, Comparisons of colony size (**a**), number of ovigerous females (**b**), skewness of colony size (**c**), and eusociality index (**d**) between social organization were performed using Bayesian phylogenetic mixed models. Error bars indicate standard errors. Brackets indicate significant differences between groups (P_{MCMC}: $***P < 0.0001$, $**P < 0.01$, $*P = 0.02$; $n = 21$).

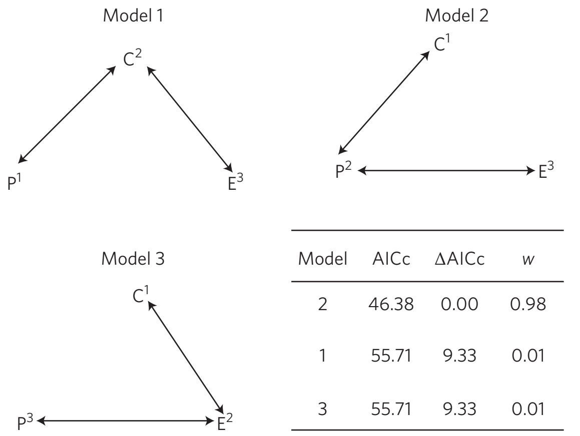
First, we defined the social organizations of *Synalpheus* species quantitatively using demographic data and then determined if these different forms of social organization vary continuously or discretely. To determine how sociality evolved in this group and to explore the subsocial and parasocial hypotheses, we then tested different models examining the evolutionary transitions among the different forms of social organization. Ultimately, this study not only provides what is to our knowledge the first empirical test outside of insects of whether the evolutionary path towards eusociality passed directly from heterosexual pairs or involved a low-skew (communal) intermediate, but it also gives us new insights into whether animal social organization evolved continuously or discretely[10].

Results

Demographic clustering. Previous treatments of sociality in *Synalpheus* informally and qualitatively suggested that there are three forms of social organization[37]. To explore and more rigorously quantify variation in social organization in *Synalpheus*, we used cluster analysis with different combinations of demographic variables (that is, colony size (CS), the skewness of colony size, the number of ovigerous females (NOF) per colony, and eusociality index (E) that quantifies reproductive skew[11,38]). We confirmed that *Synalpheus* shrimp species naturally cluster into three distinct social categories that correspond to the social organizations of pair-forming, communal breeding, and eusociality (Fig. 1 and Supplementary Table 3). Although the majority of species (22 species) are unambiguously clustered into one of these three forms of social organization (Fig. 1c,d), there were nine 'intermediate' species that clustered into different categories depending on the demographic variables used (Supplementary Table 4 and Supplementary Methods).

The unambiguously clustered pair-forming, communal and eusocial species each exhibit very different demographic characteristics in all variables measured, except for the number of ovigerous females (phylogenetically informed Bayesian regression models, all models were significant with a difference in deviance information criterion at (ΔDIC) > 8; all post-hoc comparisons had P_{MCMC} (P-value based on Markov chain Monte Carlo sampling) < 0.02 and non-overlapping 95% credibility intervals; Fig. 2 and Supplementary Table 5). In general, species with small colony sizes (CS < 8) cluster as pair-forming (note that all pair-forming species have a modal colony size of 2), species with both larger colony sizes and many ovigerous females (CS ≥ 8 and NOF ≥ 3) cluster as communal, and species with large colony sizes but few ovigerous females (CS ≥ 8 and NOF < 3) cluster as eusocial. However, the number of ovigerous females per colony did not differ between pair-forming and eusocial species ($P_{MCMC} = 0.30$); this is because a single female often monopolizes reproduction in eusocial species, despite their large colony sizes (Fig. 2).

The difference between the three forms of social organization is also apparent in bivariate relationships between demographic



Model	AICc	ΔAICc	*w*
2	46.38	0.00	0.98
1	55.71	9.33	0.01
3	55.71	9.33	0.01

Figure 3 | Comparison of three models of continuous social trait evolution among pair-forming (P), communal (C) and eusocial (E) species. The best supported model (model 2) is strongly preferred and suggests that eusociality evolved from a pair-forming state without a transition via communal breeding. The analysis was based on 22 species that were unambiguously clustered into three social organization types. The table shows the model comparison results based on the Brownian motion (BM) model, which was best supported among six different evolutionary processes. AIC, Akaike information criterion; *w*, Akaike weight. Superscripts show how we coded social organizations as continuous traits using ordered integers.

variables (Fig. 1c,d): pair-forming, communal and eusocial species appear to occupy distinct regions of the parameter space formed by colony size, number of breeding females per colony, and reproductive skew. However, *Synalpheus* species do appear to fall along a continuous axis of reproductive skew, as indicated by the eusociality index (Fig. 1d). In summary, social organization in *Synalpheus* shrimps can be classified into three demographically distinct clusters, although there are a few species that exhibit intermediate characteristics depending on the traits used in the clustering analyses.

Social transitions. Consistent with the family-centred subsocial model of social evolution, we found that the best-supported social transition model (model 2 in Fig. 3) predicts that eusocial species arose directly from pair-forming species but not from communal species. Using the 22 species that were unambiguously clustered with the demographic variables, we constructed three alternative models of continuous trait evolution (Fig. 3) and tested which model was most supported given the best current estimate of the evolutionary history of *Synalpheus*[39]. We coded social organization as ordered integers (for example, 1, 2 and 3) to model both the discreteness and continuity in their demographic characteristics (Fig. 1c,d) and to allow gradual evolutionary changes between social categories. Importantly, the best transition model (model 2) had the highest support under all six different specifications of trait evolutionary processes (Supplementary Table 6). The transition of pair-formers directly into eusocial species was consistently supported even when intermediate species were included (30 species; Supplementary Fig. 1 and Supplementary Table 7) or when species with low sample sizes (<6 colonies)—and therefore low certainty about their demographic parameters—were included (39 species; Supplementary Table 8) (see Supplementary Methods). When the intermediate species were included in the analysis, the best model further suggested that the two species that have demographic characteristics intermediate between communal and eusocial species (that is, *S. brooksi* and *S. elizabethae* that have multiple breeding females per colony; intermediate 2 in Fig. 1c,d) are derived from eusocial rather than from communal species (Supplementary Methods). Finally, mapping the discrete measures of social organization onto the *Synalpheus* phylogeny revealed the same social transition pattern (Fig. 4): eusocial species only occur in clades with pair-forming species, except for one case in which a pair-forming species (*S. ruetzleri*) has a communal and a eusocial sister species. In summary, eusociality appears to have arisen via the subsocial model in *Synalpheus* shrimps. Although communal species have intermediate levels of reproductive skew and are characterized by other demographic traits that are intermediate between pair-forming and eusocial species, the evidence supports the conclusion that communal and eusocial species are alternative

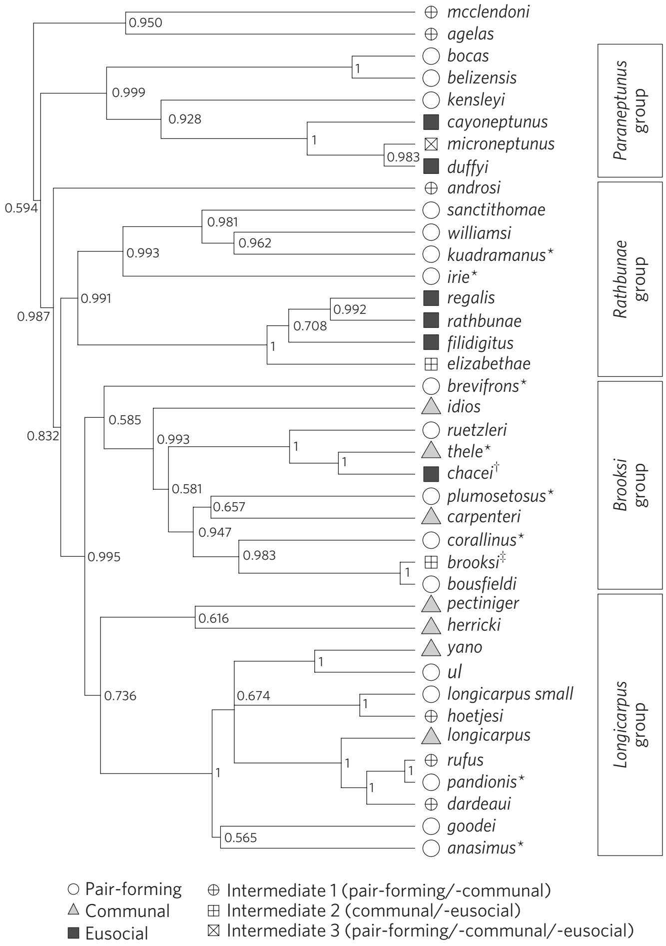


Figure 4 | Phylogeny of social evolution in *Synalpheus*. Bayesian consensus tree, constructed with 16S, 18S and COI sequence data and ultrametrized into a clocklike phylogeny. Symbols at the tips represent social organizations assigned from PAM analyses. Species with an asterisk have less than six colonies so that social organizations were inferred from Fig. 1b. Numbers at nodes represent Bayesian posterior probability values. Boxes on the right indicate the four major clades in the *gambarelloides* species group of *Synalpheus.* In the *paraneptunus* and *rathbunae* groups, eusocial species occur within more inclusive clades of pair-forming species. In the *longicarpus* group, communal species occur within a more inclusive clade of pair-forming species. In the *brooksi* group, although the eusocial species *S. chacei* (†) has a communal sister species (*S. thele*), these two species together had a pair-forming sister species. The most parsimonious explanation is that they both evolved from pair-forming ancestors, which agrees with the best-supported social trait evolution model (Fig. 3). Also in the *brooksi* group, although *S. brooksi* (‡) has mixed demographic characters of communal and eusocial species, it evolved within a clade of pair-forming species, and thus agrees with the model that intermediate 2 are derived from eusocial species (Supplementary Fig. 1).

endpoints that evolved directly and independently from pair-forming species along different evolutionary paths.

Discussion

Identifying the evolutionary path towards eusociality across different lineages can help elucidate whether different structures of animal societies represent discrete or continuous states of social organization[10]. It is especially important to clarify the role of communal species in such evolutionary paths because this form of social organization appears to be more common in both insects and vertebrates than once believed[1]. In the socially diverse snapping shrimp genus *Synalpheus,* we found strong support for the evolution of eusociality via the subsocial route that emphasizes the importance of close kin relations in the evolution of advanced sociality (through accumulation of adult offspring of a single mated pair) using a purely demographic analysis without measuring genetic relatedness[15,30]. This conclusion is consistent with a previous examination of life history traits in *Synalpheus* that showed that all eusocial *Synalpheus* species have non-dispersing larvae[11], a probable pre-adaptation to eusociality that may enable the formation of family groups, whereas most non-eusocial *Synalpheus* species have dispersing larvae that do not allow for the accumulation of kin within a sponge.

Our analyses also showed that *Synalpheus* species naturally cluster into relatively discrete groups of pair-forming, communally breeding and eusocial species, with distinct patterns of evolutionary origin. That is, eusocial and communal *Synalpheus* species each evolved directly and independently from pair-forming species. This pattern of social evolution suggests that communally breeding species do not represent an intermediate form of social organization between pair-forming and eusocial species. Hence, communal *Synalpheus* species are unlikely to have evolved into eusocial species, as suggested by the parasocial hypothesis. Importantly, our results affirm the generality of the subsocial evolution of eusociality initiated by the formation of closely related family groups as observed among both invertebrates and vertebrates[15,40–42].

Whether the forms of social organization observed in shrimp and other animal taxa form a continuum of reproductive options or represent discrete social categories that evolve independently has been a long-standing debate[8,15,22]. Our results are consistent with elements of both hypotheses. First, *Synalpheus* species vary uniformly along a continuous scale of reproductive skew (eusociality index, Fig. 1d). Second, other demographic traits that describe social organization in *Synalpheus* species also vary continuously, particularly between pair-forming and communal species (Fig. 1c). Yet, although these demographic characteristics suggest that *Synalpheus* species may form a single continuum, our analysis of their evolutionary history suggests that the evolution of the different forms of social organization along this continuum can take one of two distinct directions. Specifically, communal species evolve from pair-forming species along one continuum, and eusocial species evolve from pair-forming species along another, non-overlapping continuum. This divergence in the demographic traits underlying social organization is consistent with the hypothesis that eusociality and communal breeding represent distinct social states characterized by different patterns of reproductive skew[10,13]. This further implies that, despite continuous variation in reproductive skew among species with different forms of social organization, reproductive skew arises for different reasons in communal and eusocial species because eusocial species evolve by the retention of offspring and communal species do not. Thus, caution should be taken when analysing social species along a continuum of reproductive skew, as it may obscure patterns that operate separately along alternative social paths.

Finally, our findings support the idea that eusociality and communal breeding represent alternative stable states in social evolution[10], and may be different solutions to similar environmental pressures such as nest or resource limitation[13]. Several benefits of communal breeding in defense and energetic savings have been proposed[13], but these ideas remain to be tested empirically. In *Synalpheus*, the prevalence of communal species (at least 7 communal versus at least 6 purely eusocial species (and 3 intermediate species that are typically defined as eusocial) out of the 39 *Synalpheus* species in Fig. 4) and the fact that they evolved independently multiple times suggests that communal breeding and eusociality may both offer strong selective advantages. In fact, communal and eusocial *Synalpheus* species often occupy the same individual host sponge, which suggests that these social strategies may be similarly competitive. A recent synthesis also found that communal societies with relatively low skew are more common in social insects and vertebrates than once thought, and called for more work in communal species to understand social evolution[1]. Our study shows that communal species are prevalent and have evolved separately from eusocial species in shrimp. Since eusocial and communal species have different patterns of dispersal that result in very different kin structures[11,35], they are likely to be influenced by very different sets of selection pressure, despite the fact that these types of species often co-occur in the same sponge host. That is, kin selection is likely to play a fundamental role in the evolution of eusociality, but not in communal breeding. Therefore, the diversity of forms of social organization in *Synalpheus* shrimps offer an interesting system within which to clarify the role of kin selection in social evolution, something that will require more work.

Whether variation in animal social organization should be characterized discretely or continuously by patterns of reproductive skew depends on the evolutionary transitions between pair-living, communally breeding and eusocial societies. We showed that social evolution in the snapping shrimp genus *Synalpheus* involved two distinct paths in which communal breeding and eusociality represent alternative evolutionary endpoints, potentially with different fitness benefits that are likely to be selected for by different processes[13]. Furthermore, despite living in a very different marine ecosystem than all other eusocial animals, eusociality in *Synalpheus* appears to have a similar 'family-centred' origin, which affirms the generality of close genetic relatedness in the evolution of advanced animal societies.

Methods

Collections. We collected sponges and their associated macrofauna from shallow habitats in eight countries in the tropical West Atlantic from 1988 to 2014 (see Supplementary Table 1 for details). In general, we collected either macroscopic sponges attached to hard substrates or cryptic sponges attached to or infilling between dead coral rubble using SCUBA (5–20 m) and snorkelling (<5 m). Sponges were collected whole and kept submerged in seawater during transportation and until processing at field stations. Sponges were then dissected and all macrofauna were carefully removed from the internal canals of the sponge. We sorted *Synalpheus* shrimps by species and counted the number of ovigerous (egg-bearing) females and non-ovigerous individuals in each sponge. Non-ovigerous individuals can be female or male[34], but they cannot be sexed in the field. All shrimp of the same species from the same sponge were considered a colony. Shrimps were preserved in 95% EtOH. *Synalpheus* identification was based on recent taxonomic descriptions and keys (for example, ref. [43]). Uncertain specimen identifications were confirmed using COI and 16S sequences and established phylogenies[44].

Demographics. The social organizations of *Synalpheus* species are often noted qualitatively in taxonomic descriptions (for example, ref. [43]), but there has been no attempt to quantitatively define discrete forms of social organization in *Synalpheus* using a defined set of life history traits. To provide a quantitative classification of social organization, we used demographic traits of 31 *Synalpheus* species within the *S. gambarelloides* group collected from the tropical West Atlantic ($n = 1{,}233$ unique colonies; Supplementary Tables 1 and 2). We quantified colony size (CS) as the total number of individuals in a sponge, and the number of ovigerous females (NOF) for each colony from each sponge. After examining the distributions of CS and NOF among species, we also calculated the skewness of CS and NOF because skewness (among the four central moments) captured the strong asymmetries of CS and NOF that are apparent in some species (Fig. 1d). These variables were analysed using a log-2 scale, which better describes geometric population growth. As an alternative single measure that summarizes both colony size and the number of ovigerous females simultaneously, we calculated the eusociality index (E), a modified version of Keller and Perrin's eusociality index[38], as $E = 1-((2 \times \text{NOF})/\text{CS})$ *sensu*[11]. The eusociality index describes the number of ovigerous females relative to colony size (that is, reproductive skew[8]), making the simplifying assumption that all breeding individuals contribute equally to offspring production.

***Synalpheus* phylogeny.** Morphological and molecular characters for several new species were added to previously published data sets to create our *Synalpheus* phylogeny[44]. Molecular data consisted of three loci: the mitochondrial 16S rRNA locus (16S), the 5' barcoding end of the mitochondrial cytochrome oxidase I gene (COI), and a region of the 18S nuclear large ribosomal subunit (18S). Collection locations, voucher locations and taxonomic information are summarized in Supplementary Table 9. DNA extraction, primers, amplification, sequencing methods and sequence analyses have been described previously[44]. We used GBlocks v0.9 (allowed gap positions = half) to exclude ambiguous parts of the alignment for 16S and 18S[45], resulting in useable regions of 446 bp for 16S and 663 bp for 18S; the COI region used was 669 bp. Finally, we used MrModelTest v2.3[46] to code the general model of evolution for each locus (COI and 16S: GTR+I+G, rates = invgamma, 18S: K80+I+G, rates = invgamma). In addition to the sequence data, we used a set of 33 morphological characters, compiled (with slight modifications) from two previous published data sets[47,48]. Morphological characters for the new species were scored by K.M.H.

We ran a partitioned Bayesian analyses in MrBayes v3.2.5[49]. Although we were missing data for 1–3 species for each set of data (COI, 16S, 18S, morphological data; Supplementary Table 2) because some regions failed to amplify, we opted to utilize all taxa with data for at least 2 of the 3 loci and treated all gap data as missing data. This was based on a preliminary analysis of our data set and simulations suggesting inclusion of such taxa improved the accuracy of the final tree[50]. We ran Markov Chain Monte Carlo (MCMC) searches with four chains and two runs for 2×10^7 generations, sampling the chain every 1,000 generations. For all trees, we discarded the first 25% (standard deviation of split frequencies after this burn-in sample ≤ 0.01), and estimated support for nodes using Bayesian posterior probabilities (bpp).

We converted the tree into a clocklike phylogeny by estimating evolutionary rates using penalized likelihood and verified the rates by cross-validation[51]. We fixed the age of the root to one and scaled the tree by the absolute rate using chronopl in the *R* package *APE*[52]. We used this ultrametric tree for testing trait evolution using Bayesian phylogenetic mixed models[53].

Demographic clustering. We focused the analysis within the monophyletic gambarelloides species group[44] and on species for which we had samples of at least 6 different sponges. To obtain accurate demographic variables, we excluded all partially sampled sponges and colonies with a single individual or with no ovigerous females. Demographic variables included colony size, the number of ovigerous females, skewness of CS and NOF (skCS and skNOF) for each colony from each sponge, and eusociality index[11,38]. To identify natural clusters of *Synalpheus* species based on demographic properties, we employed the Partitioning around Medoids (PAM) algorithm[54] using the *R* package *cluster*[55]. This algorithm clusters objects about k medoids and minimizes the sum of the distances (Silhouette distance, s_i) from each object to the closest medoid[54]. To avoid subjective selection of input variables, we partitioned *Synalpheus* species using seven combinations of five normalized variables: CS, NOF, skCS, skNOF and E (Supplementary Table 4). Each combination had at least CS and NOF or E as the main variables. Combinations with E did not include CS and NOF because E is a composite trait calculated from these two variables (Supplementary Table 3). For each combination of input variables, we ran the algorithm separately with 2 to 6 clusters (k), and then determined the best k value as the one that has the highest average silhouette distance (s_i) among the seven analyses with different input variables. The silhouette distance measures how well an object fits into its own cluster rather than the nearest neighboring cluster[56], and so the highest silhouette distance means the most discrete clustering. An average silhouette distance above 0.5 indicates that a reasonable structure has been found[56] (such as for $k = 3$ in Fig. 1a). After selecting the best number of clusters, we identified the species that always clustered into the same group among all seven analyses (hereafter 'unambiguous species'), and species that did not have a consistent group assignment that were clustered into different groups in different analyses ('intermediate species').

To explore how demographic metrics contributed to clustering of the unambiguous species, we built a classification tree[57] using CS, NOF, skCS and skNOF with the *R* package *rpart*[58]. The *rpart* algorithm performs recursive partitioning to create decision rules for predicting a categorical outcome. According to the criteria of the classification tree, we assigned *post hoc* groupings to 18 species with less than 6 colonies; these species were only used for supplementary analysis of social transitions.

The PAM analyses suggested that *Synalpheus* species naturally clustered into three groups, which conformed to pair-forming, communal and eusocial categories that were based on the demographic characteristics (see Results for more details). We explored differences in demographic and social traits among 21 species that were unambiguously clustered into these three categories, excluding the 'intermediate' species. First, we used Bayesian phylogenetic mixed models in the *R* package *MCMCglmm*[53] to test whether the variables NOF, CS, skCS and E differed among groups. We checked for normality visually and with the Shapiro-Wilk normality test, and log-transformed skCS and square-root transformed NOF. We used the standard inverse-gamma (0.001, 0.001) distribution as the prior distribution for the residual variance and the variance components (that is, random effect based on the phylogeny). Model significance was assessed using deviance information criteria (DIC) against a null model[59]. *Post hoc* comparison between the different forms of social organization were assessed by testing whether the 95% credibility intervals (CI) overlapped zero, or by P_{MCMC} values.

Social transitions. We investigated the transitions among 22 unambiguously clustered species with different forms of social organization (that is, pair-forming, communal and eusocial) by mapping the distribution of social organizations onto the *Synalpheus* phylogeny and reconstructing transitions among social states. Our primary goal was to test whether eusociality evolved directly from pair-forming species or via communal species as an intermediate. We coded the forms of social organization as continuous traits in various configurations of ordered integers from 1 to 3 following three different social transition models (Fig. 3). We treated social organization as a continuous rather than a discrete trait because species showed continuity in their demographic characteristics (Fig. 1c,d) and to allow gradual, instead of abrupt, change between social categories. The coding configurations allowed directional transitions between social organizations coded as adjacent integers (for example, with social organizations coded as 1 to 3, direct transitions were allowed between 1 and 2, and 2 and 3), but prevented the direct transition between the two social organizations coded as non-adjacent integers (for example, 1 and 3). We tested the fit of these competing social transition models using fitDiscrete in the *R* package *geiger* v2.0.3[60]. In addition, because the process underlying trait evolution was unknown, we modelled six different evolutionary processes: Brownian motion (BM), BM+λ, BM+κ, Ornstein-Uhlenbeck (OU), ACDC, and white noise (reviewed in ref. [61]). The BM process assumes a Brownian motion of continuous trait evolution. The additional parameters λ and κ estimate the amount of phylogenetic signal and a degree of branch length transformation conforming to a punctuational model of evolution, respectively. The OU process assumes stabilizing selection, and the ACDC process assumes adaptive radiation in which character evolution rate can accelerate or decelerate. The white noise process assumes that characters evolved at random, independent of the phylogeny. To maximize the chance of finding the optimal solution in the likelihood space, we ran each model with 1,000 random starting points. Finally, we compared the resulting 30 models with Akaike information criterion adjusted for small sample sizes (AICc)[62]. Although the *Synalpheus* phylogeny contains a polytomy, analyses with all possible resolved trees yielded identical results. We excluded *S. microneptunus* in this analysis because it was assigned to all three forms of social organization. Excluding *S. microneptunus* did not affect the analysis because it is nested within a eusocial clade (*S. duffyi* and *S. cayoneptunus*; Fig. 4) and is likely to have had a eusocial ancestor. We performed two supplementary analyses to (i) use unambiguously clustered species and intermediate species, and (ii) include species with *post hoc* assignments of social organizations (Supplementary Methods). Results from these analyses were reported in Supplementary Tables 7 and 8.

Data availability. The *Synalpheus* demographic data generated and analysed during this study are included in this Article and its Supplementary Information. Sequence data generated in this study have been deposited in GenBank (Supplementary Table 9).

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Author contributions

S.T.C.C., J.E.D., K.M.H. and D.R.R. collected field samples. S.T.C.C. carried out the statistical analyses and drafted the manuscript; S.T.C.C., J.E.D., K.M.H. and D.R.R. conceived of the study, designed the study, coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

Additional information

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Correspondence and requests for materials should be addressed to S.T.C.C.

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Competing interests

The authors declare no competing financial interests.