

Research



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Phylogeny of the carpenter bees (Apidae: Xylocopinae) highlights repeated evolution of sociality

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Many groups of animals have evolved social behaviours in different forms, from intimate familial associations to the complex eusocial colonies of some insects. The subfamily Xylocopinae, including carpenter bees and their relatives, is a diverse clade exhibiting a wide range of social behaviours, from solitary to obligate eusociality with distinct morphological castes, making them ideal focal taxa in studying the evolution of sociality. We used ultraconserved element data to generate a broadly sampled phylogeny of the Xylocopinae, including several newly sequenced species. We then conducted ancestral state reconstructions on the evolutionary history of sociality in this group under multiple coding models. Our results indicate solitary origins for the Xylocopinae with multiple transitions to sociality across the tree and subsequent reversals to solitary life, demonstrating the lability and dynamic nature of social evolution in carpenter bees. Ultimately, this work clarifies the evolutionary history of the Xylocopinae, and expands our understanding of independent origins and gains and losses of social complexity.

1. Introduction

The evolution of eusociality has fascinated biologists for decades, and bees have served as the focus of much research on this topic. Sociality has evolved numerous times in bees, with the most derived and complex behaviours demonstrated in the corbiculates (Apidae: Apinae) [1,2]. However, bees collectively exhibit a wide spectrum of behavioural strategies, and social behaviour is also seen in other groups including sweat bees (Halictidae; [3]) and carpenter bees (Apidae: Xylocopinae; [4]).

Xylocopines in particular demonstrate a higher degree of social flexibility than most other bee taxa (comparable to the social diversity of Halictidae), making them an excellent focal group to study evolutionary transitions between various forms of sociality. The subfamily Xylocopinae includes the full spectrum of sociality from solitary bees such as *Manuelia* [5] to eusocial species, like many members of the tribe Allodapini [6,7]. Some species within the Xylocopinae also demonstrate variable social behaviour with solitary and social colonies in sympatry making them excellent models to examine the costs and benefits of sociality [8].

Previous work on this group has suggested that it derives from an ancestrally social origin, with multiple independent transitions to solitary living, and a few cases of progression to more complex sociality [4]. However, subsequent developments in phylogenetic research have suggested alternate systematic relationships within the Xylocopinae which may change this inference [9]. Under this new definition, the subfamily Xylocopinae includes six tribes. The monogeneric Ceratinini and Xylocopini each include about 370 species, while the Allodapini includes most of the remaining xylocopines with about 280 species [10]. The tribe Manuelliini includes just three species, and along with the Xylocopini, Ceratinini and Allodapini constitutes the historical definition of Xylocopinae (henceforth 'Xylocopinae *sensu stricto*'). Nearly all members of Xylocopinae *sensu stricto* build nests in wood or plant stems, either

by excavating material themselves or making use of pre-existing cavities, although a small number of species are ground-nesting or may occasionally use other substrates [11]. The two additional tribes added to Xylocopinae by Bossert *et al.* [9], Ctenoplectrini and Tetrapediini, contain about 45 species between them [10]. Both of these tribes include oil-collecting bees which nest in pre-existing cavities in wood.

Here we reconstruct the phylogeny of Xylocopinae including all six currently recognized tribes, using expanded ultraconserved element data and newly sequenced taxa to generate a more robust dataset for this clade than any previously available. The objective of this study is to revisit the evolutionary origins and elaborations of social behaviour in the Xylocopinae, with broader implications for the evolution of eusociality in arthropods more generally.

2. Methods

(a) Ultraconserved element data collection

Previously published ultraconserved element (UCE) contigs were obtained for 166 species of Xylocopinae and 10 outgroup taxa ([9,12–17]; see electronic supplementary material, table S1 for the full list). For an additional three species, UCE contigs were extracted from whole genome assemblies produced by Shell *et al.* [18]. This was accomplished using the ‘phyluce_probe_slice_sequence_from_genomes’ script within Phyluce [19].

Additionally, new UCE sequence data were generated for six specimens: *Eucondylops konowi*, *Exoneurella eremophila*, *Exoneuridia oriola*, *Hasinamelissa curtipilosa*, *Tetrapedia diversipes* and *Xylocopa aerata* (electronic supplementary material, table S1). DNA was extracted from these following a standard non-destructive sampling protocol [20] and was then sent to Arbor Biosciences for library preparation, enrichment, and sequencing, using the Hymenoptera v2 ‘ant-bee’ probe set [21]. The resulting reads were assembled into contigs with SPAdes v3.15.4 [22].

(b) Phylogenetic reconstruction

The Phyluce pipeline [19] was used to extract, trim, and align UCE loci from all contigs to create a final alignment. Specifically, the probe set described above was used to identify UCE loci within contigs, which were then aligned using MAFFT [23] and trimmed with Gblocks [24]. This alignment was then filtered to include only loci present in at least 90% of included taxa. A final quality control step was carried out with SpruceUp [25] using the uncorrected distance method, a window size of 50 bp with 25 bp overlap, and cut-off of 0.98. UCE loci were partitioned according to the Sliding-Window Site Characteristics Entropy (SWSC-EN) method implemented in PFinderUCE [26].

The initial phylogeny was constructed using IQ-TREE v2.0.7 [27], using the partition file provide by PFinderUCE and with ultrafast bootstrap [28] and SH-aIrt [29] metrics to assess branch support. The topology of this tree was compared to other phylogenies of Xylocopinae and was ultimately regenerated using a constrained topology at the node corresponding to the *Ceratina* subgenus *Pithitis* (IQ-TREE ‘-g’ option) due to incongruence with other trees with more complete taxon sampling ([4,30], unpublished data).

(c) Ancestral state reconstruction

A review of the literature was used to assess the behaviour of as many included xylocopine taxa as possible, resulting in three coding schema (electronic supplementary material, table S1):

1. In the simplest two-state model, as per Rehan *et al.* [4], taxa were coded as either ‘solitary’, if they had only been recorded with a lone female in reproductive nests, or ‘social’ if any records indicated some form of cooperative breeding (regardless of prevalence or relatedness). Specifically, species were considered social if multiple adult females were recorded in a nest while brood were being reared. Subsocial species (with extended care of adult offspring but no simultaneous rearing of brood) were thus included under the ‘solitary’ category.
2. In the three-state model, the ‘social’ category above was subdivided into ‘facultatively social’ species, in which both social and solitary behaviours were recorded (regardless of relative frequency), and ‘obligately social’ species known to exclusively nest socially.
3. Finally, in the four-state model, the above categories were retained, and the ‘solitary’ group was additionally subdivided between fully solitary species and ‘subsocial’ species with confirmed evidence of extended maternal care of adult young and overlapping generations (but no cooperative brood care by other females; [31]).

Ancestral state reconstructions were carried out using the R package phytools [32]. The ‘make.simmap’ function was used, with the model set to ‘all rates differ’ and 1000 simulations (see electronic supplementary material, table S2 for full details). Each reconstruction was carried out twice, first using the full phylogeny including all taxa, and again on a pruned phylogeny retaining only those taxa which could be scored for social behaviour.

3. Results

(a) Alignment statistics and phylogenetic relationships

The final alignment included 185 taxa (175 species of Xylocopinae and 10 outgroups), and consisted of 417 UCE loci with a total length of 84 384 bp and 7.24% missing data. The tribe-level relationships recovered in the tree (figure 1) place Ctenoplectrini and Tetrapediini together as the sister group to the four tribes of Xylocopinae *sensu stricto*. Within this latter group, Manueliini + Xylocopini jointly form the sister group to Allodapini + Ceratinini. Most nodes in the full tree were robustly supported according to branch support metrics, and all six tribes were recovered as monophyletic (electronic supplementary material, figure S1). Relationships between tribes were also all fully supported with the exception of the node uniting Ctenoplectrini and Tetrapediini, which received 36/74 support under ultrafast bootstrap and SH-aIrt metrics respectively.

(b) Evolution of sociality

Ancestral state reconstructions were carried out on the phylogeny using the two-, three- and four-state character coding schema described above. In total, 72 out of 175 ingroup taxa were coded (41.1%; electronic supplementary material, table S1). The reconstruction results from the pruned phylogeny including only these 72 taxa were overall very similar to those obtained from the full phylogeny including taxa with unknown behaviour. The following results refer to the former dataset except where noted (for reconstruction results on the full dataset see electronic supplementary material, tables S2 and S3).

The most recent common ancestor of Xylocopinae was recovered as solitary with high probability under all models

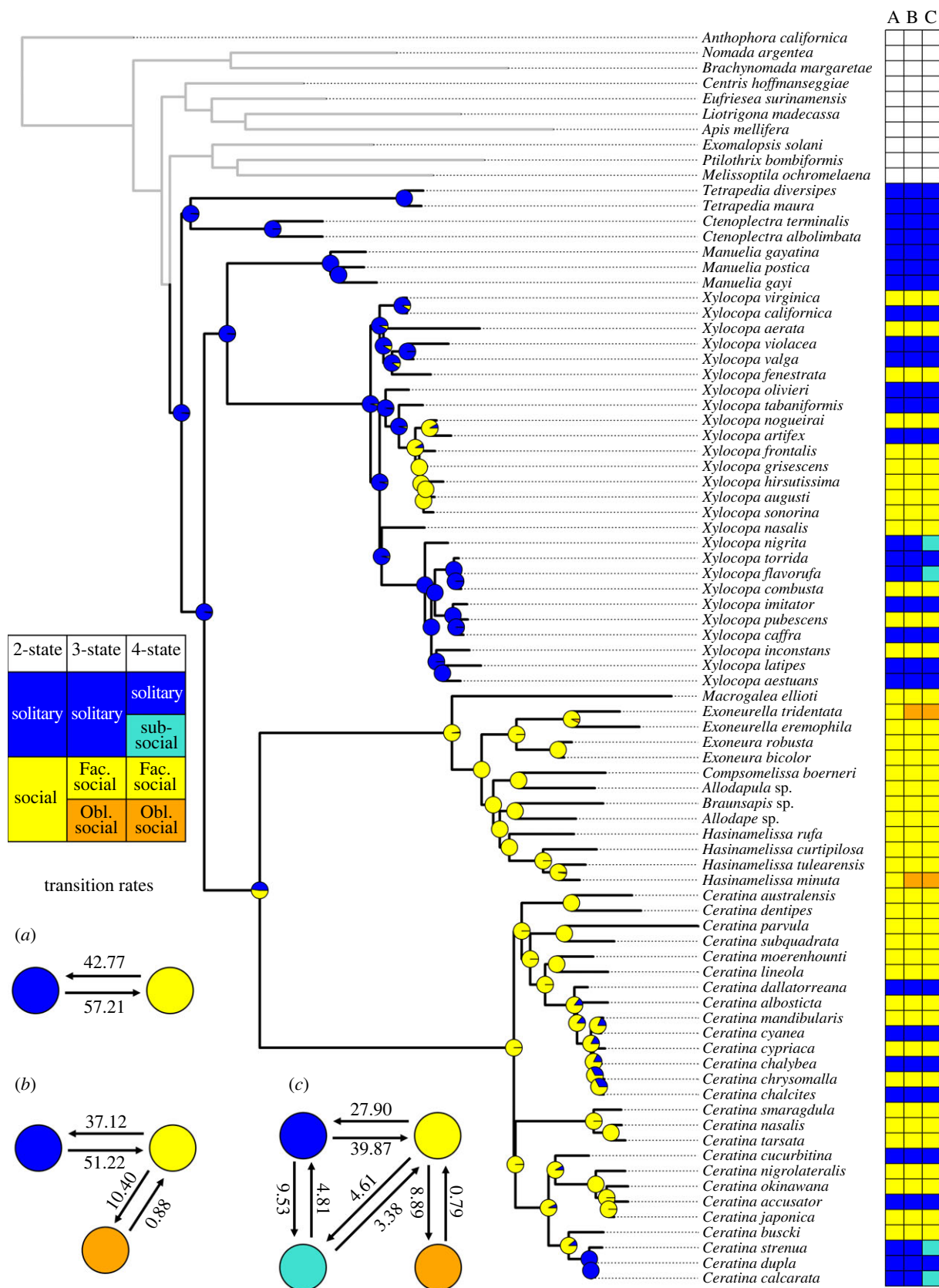


Figure 1. Pruned phylogeny of Xylocopinae showing the evolution of social behaviour. Boxes to the right show codings for each taxon under the two-state (a), three-state (b) and four-state (c) models respectively, following the key on the left. Pie charts at nodes show reconstructed probabilities of behavioural states under the three-state model. Proportional transitions in bottom left are shown for the two-state (a), three-state (b), and four-state (c) models. Transitions accounting for less than 0.5% of totals are excluded.

(prob. 0.987, 0.991 and 0.980 under the two-, three- and four-state models respectively; electronic supplementary material, table S2). The node representing Xylocopinae *sensu stricto* showed similar results, with solitary living again being

recovered as most probable under all models (prob. 0.977, 0.983 and 0.970, respectively). The solitary state was also recovered as most probable under all models for the tribes Manueliini (prob. 1.000 under all models), Xylocopini

(prob. 0.971, 0.970, 0.960), and the clade uniting the tribes Ctenoplectrini and Tetrapediini (prob. 0.989, 0.992, 0.985). By contrast, the social (two-state model) or facultatively social (three- and four-state models) states were recovered as ancestral for the tribes Allodapini (prob. 0.987, 0.983, 0.984) and Ceratinini (prob. 0.997, 0.996, 1.000). However, the ancestral state of the node uniting Allodapini and Ceratinini was more ambiguous, with the social/facultatively social state being marginally more likely than the solitary states under the two- and three-state models with the pruned dataset (prob. 0.537, 0.509) but the solitary state receiving slightly higher probability for the four-state model (0.487) and all models using the full, unpruned dataset (prob. 0.536, 0.538, 0.518).

Under the two-state model, transitions from solitary to social behaviour accounted for 57.21% of transitions across all simulations, with the reverse direction accounting for the remaining 42.77% (electronic supplementary material, table S3). The three-state model was similar, with 51.22% of all transitions being from solitary to facultatively social (37.12% in reverse direction), as well as 10.40% of transitions going from facultative to obligate sociality, with negligible back-transitions from the obligate social state to the solitary (0.37%) and facultative social (0.88%) states respectively. The four-state model was more complex, though again showed most transitions occurring between the solitary and facultatively social states (39.87% forward, 27.90% reverse), as well as a moderate proportion of transitions from solitary to subsocial (9.53%, reverse 4.81%) and from facultatively social to obligate social (8.89%, reverse 0.79%).

Considering the ancestral state reconstructions across the tree as a whole, social behaviour is inferred to have evolved on several occasions, with at least three predominantly social clades. Facultative sociality is widespread in one clade within the *Xylocopa* subgenus *Neoxylocopa*, as well as several individual *Xylocopa* species from other subgenera. Facultative sociality is also ancestral for both the tribes Ceratinini and Allodapini (though it is unclear if this represents a single shared origin or two independent transitions), with the *Ceratina* subgenus *Zadontomerus* showing a reversal to solitary life, as well as various isolated reversals in other species. The Allodapini do not show any reversals to the solitary state, but subsequently evolved obligate sociality in two known species (*Exoneurella tridentata* and *Hasinamelissa minuta*).

4. Discussion

(a) Evolution of sociality in Xylocopinae

Here we revisited the evolution of sociality in Xylocopinae in light of the inclusion of additional wood-dwelling and oil-collecting solitary bee tribes near the root of this group as suggested by recent phylogenetic research [9]. These results differ somewhat from Rehan *et al.* [4], who also investigated this question and suggested social origins for Xylocopinae (*sensu stricto*). With our expanded phylogeny of Xylocopinae, we instead recover solitary origins for the subfamily under all coding schema tested. The implication of this finding is that, rather than a single social origin with repeated transitions to solitary living, the carpenter bee clade has evolved dynamically from its solitary ancestry, with multiple origins of social behaviour and reversals to solitary life.

These results are generally consistent across the three coding schema tested. The most straightforward two-state model clearly supports this principal result of a solitary origin for Xylocopinae. This is corroborated by the three-state model, which also provides the additional context of the more complex social behaviours which evolved in the Allodapini, such as the evolution of morphologically differentiated castes [7,8]. The additional distinction of subsociality in the four-state model provides an additional layer of nuance, though the accuracy of this model is also influenced by the requirement for more detailed observations which are lacking in many species, meaning that subsociality may be underreported in carpenter bees and related taxa.

The frequency of transitions to and from social behaviour occurring in the Xylocopinae *sensu stricto* may be supported by several potential preadaptations to sociality, including unusually long lifespans and prereproductive assemblies of adult siblings [8]. Though such preadaptations are not yet known from the Ctenoplectrini or Tetrapediini [33,34], the inclusion of these tribes under the expanded definition of Xylocopinae further solidifies the reconstructed solitary origins of the subfamily, and additional study of these tribes could further clarify the origins of some of these potential 'pre-social' behaviours.

As a potential caveat, these findings may be influenced to some degree by the taxon sampling used to create our phylogeny. Though *Xylocopa* and *Ceratina* do account for the majority of xylocopine species, they are still somewhat over-represented in our dataset in comparison to the tribe Allodapini, for which there is a lack of available UCE data (even when including the four new species sequenced as part of this study). Since this group clearly has social origins, the inclusion of more allodapine taxa could potentially influence the reconstructed states at some nodes in this direction, especially the highly ambiguous node uniting Allodapini and Ceratinini.

The availability of high-quality UCE data for many taxa has made the creation of robust phylogenies relatively routine, with many recent studies within bees working at the family or subfamily scale. However, a major limitation in investigating the evolutionary history of behavioural traits remains the lack of detailed natural history data for many species. Future studies on the nesting biology and sociobiology of these disparate taxa are much needed. As discussed by Michener [8], many xylocopine species demonstrate flexibility in social organization, either on an individual basis or over time within a single nest; this is particularly noteworthy within the Allodapini and Ceratinini, reviewed by Schwarz *et al.* [35] and Rehan [36], respectively. The nesting biology of Xylocopinae may lend itself to sociality to some degree as well, especially in taxa such as the Allodapini which raise their young in a common space without separation into brood cells in a manner superficially similar to many ants [8]. This variation in sociality within species provides a natural experiment for future studies on the genetic bases and ecological circumstances favouring social group formation at its onset.

(b) Xylocopinae among other eusocial taxa

Independent origins of eusociality are known from a variety of arthropod taxa, including other groups of bees (most Apinae, some Halictidae; [11]) and hymenopterans

(Vespidae, Formicidae; [1]), thrips (some Thysanoptera; [37]), termites (Isoptera; [1]), and snapping shrimp (*Synalpheus*; [38]). In several of these groups, all living species are obligately eusocial, making it difficult to investigate the evolutionary origins of this highly derived behaviour due to the lack of taxa exhibiting facultative strategies. These evolutionary dynamics are further complicated by the fact that facultatively social clades may experience reversals to solitary behaviour relatively commonly [39,40], while obligately social groups may pass a 'point of no return' beyond which such reversals are very rare [41]. For this reason, clades like the Xylocopinae are instrumental to the study of sociality because they collectively represent a range of behavioural strategies which all co-occur in the present day. For example, our ancestral state reconstructions indicate that the states of being solitary and facultatively social are fairly interchangeable, with relatively high transition rates in both directions, as has also been demonstrated in halictid bees [40]. However, no transitions back to solitary life are recovered after eusociality evolved in the Allodapini, which helps to explain the occurrence of large and ecologically dominant eusocial clades such as ants, termites and corbiculate bees.

5. Conclusion

Our study presents the most detailed and complete phylogeny to date of carpenter bees and their relatives, subfamily Xylocopinae, based on ultraconserved element sequence data. We use this phylogeny to examine the fascinating and complex evolutionary history of social behaviour in this

group. Notably, our results strongly suggest solitary origins for the Xylocopinae in contrast to previous work, as well as multiple origins of social behaviour in the tribes Allodapini, Ceratinini, and Xylocopini and subsequent reversals back to solitary living. This refined interpretation of sociality in carpenter bees holds far-reaching implications for the broader understanding of social evolution and emphasizes the need for further study of other socially labile groups of arthropods and animals more generally.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. Sequence data for all newly sequenced species as part of this study are available through the NCBI SRA under bioprojects PRJNA957433 and PRJNA966956.

The data are provided in the electronic supplementary material [42].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. T.S.: data curation, formal analysis, investigation, visualization, writing—original draft; S.R.: conceptualization, funding acquisition, investigation, project administration, resources, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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