**Monophyly of eusocial wasps (Hymenoptera: Vespidae): molecules and morphology tell opposing histories**

**INTRODUCTION**

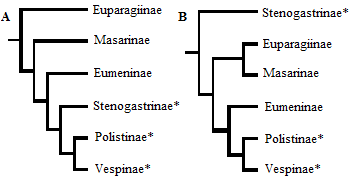
Studying the transition from solitary to eusocial life in insects has the potential to elucidate long-standing evolutionary questions regarding phenotypic decoupling and altruistic behavior. Phylogenies provide insight into the ancestral states and environmental contexts that subserve worker/queen decoupling, and thus are the framework for understanding origins of eusociality. We present a thorough phylogenetic analysis of the Vespidae, utilizing phenotypic and molecular data of previous studies [1,2] in conjunction with newly acquired data, in an effort to explain how solitary ancestors may have crossed the threshold of eusociality. Previous studies show conflicting phylogenies for Vespidae [1,2] and differ in their conclusions regarding whether eusociality has evolved once or twice in these wasps. The causes of phylogenetic incongruence are numerous and so we postulate the primary drivers of phylogenetic incongruence complicating reconstruction of the Vespidae phylogeny.

**METHODS**

Simultaneous analysis of phenotypic characters (269 morphological and 66 behavioral) and molecular data (CO1, 28S, 16S, 12S) for 74 taxa was performed to clarify the subfamily relationships of the Vespidae. Alignments were implemented using CLUSTAL W and corrected by-eye. A total of 4051 base pairs were used for all analyses, of which 1056 sites were parsimony informative. Additional analyses were performed after exclusion of only behavioral characters, and also after exclusion of all phenotypic data. To account for phylogenetic incongruence brought on by different cladistic methods, we employed Maximum Likelihood, Bayesian Inference and Maximum Parsimony for all data inclusion schemes, resulting in nine main topologies for comparison.

**RESULTS**

Simultaneous analysis of all evidence supports a (Stenogastrinae + (Polistinae + Vespinae)) clade and thus a single origin of eusociality (Figure 1A). Our results support the novel tribal relations of Polistinae proposed in a recent study [2] as (Ropalidiini + (Mischocyttarini + (Polistini + Epiponini))). Phylogenies derived strictly from molecular evidence place Stenogastrinae as basal and thus support a dual origin of eusociality (Figure 1B).

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**Figure 1.** Vespidae subfamily relationships under Maximum Parsimony derived from (A) simultaneous analysis of phenotypic and molecular data and (B) molecular data only. Asterisk denotes groups that display eusocial behavior.

**DISCUSSION AND CONCLUSIONS**

Our reanalysis shows that previous studies recovering diphyly of eusocial wasps [1] may withstand the effects of low taxon sampling, suboptimal alignments and a two-step approach causing phylogenetic error; we achieved similar topologies using ~three-fold more terminal ingroups and a different suite of loci. Also, for simultaneous analysis of all data under parsimony, our two-step regime retrieved a similar topology as a study that employed one-step direct optimization [2].

All trees derived exclusively from molecular evidence suggest dual origins of eusociality; however, the relative positions of Euparagiinae, Masarinae and Stenogastrinae are inconsistent across the different cladistic methods. Our results show that a portion of phylogenetic incongruence is attributable to method choice, but that the primary driver of incongruence is data type inclusion – that is, the topology produced changes when phenotypic data is included. We conclude that vespid subfamily relationships are obfuscated by the fact that phenotypic and molecular evidence are at odds. Nonetheless, we advocate topologies retrieved from simultaneous analysis of all available evidence. Thus, a single origin of wasp eusociality is the best hypothesis, highlighting the rarity and salience of simultaneous progressive provisioning emergence.

**REFERENCES**

1. Hines H, et al. *PNAS.* **104**:3295-3299, 2007.
2. Pickett & Carpenter. *Arthropod Syst. Phylogeny.* **68**:3-33, 2010.