

Assembling the ant "Tree of Life" (Hymenoptera: Formicidae)

Philip S. WARD, Seán G. BRADY, Brian L. FISHER & Ted R. SCHULTZ

Abstract

Ants are the world's premier eusocial organisms and they have assumed pivotal ecological roles in many terrestrial communities. A well resolved and robustly supported phylogeny of the ants is needed to better understand many facets of their ecology and evolution. We report on a new project designed to clarify the phylogenetic relationships of the major lineages of ants. Funded by the US National Science Foundation under the AToL (Assembling the Tree of Life) program, this project runs for five years and involves collaborators from the University of California at Davis, the California Academy of Sciences, and the Smithsonian Institution. In the first year (2004 - 2005) of the Ant AToL grant we have focused our efforts on developing a multi-gene molecular data set and assembling a selection of appropriate taxa. A preliminary analysis, based on ~5.8 kb of sequence data from seven nuclear genes and ~100 exemplar species, reveals a number of novel findings and contradicts some earlier conclusions derived from morphological data. Nearly all of the 21 extant ant subfamilies recognized in a recent morphology-based classification of ants (BOLTON 2003) appear to be monophyletic, but our evidence suggests that only two of the six supra-subfamilial groups (dorylomorphs and myrmeciomorphs) are monophyletic. We find strong support (parsimony and likelihood bootstrap 100 %, Bayesian posterior probability 1.00) for a group, here termed the "formicoid clade", which contains all extant ants except Agroecomyrmecinae, Amblyoponinae, Leptanillinae, Paraponerinae, Ponerinae, and Proceratiinae. Relationships among these early diverging (non-formicoid) ants are not well resolved. This work is ongoing, and a more comprehensive account and analysis with additional taxa and new molecular data will be completed at the year's end.

Key words: Formicidae, phylogeny, molecular systematics, evolution, social insects

*Prof. Dr. Philip S. Ward (contact author), Department of Entomology, University of California, Davis, CA 95616, USA.
E-mail: psward@ucdavis.edu*

Dr. Seán G. Brady, Department of Entomology and Laboratories of Analytical Biology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560, USA.

Dr. Brian L. Fisher, Department of Entomology, California Academy of Sciences, 875 Howard Street, San Francisco, CA 94103, USA.

Prof. Dr. Ted R. Schultz, Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560, USA.

Introduction

Ants (Hymenoptera: Formicidae) are the world's most successful group of social insects, with a diversity that probably exceeds 20,000 species (HÖLDOBLER & WILSON 1990). They have a strong ecological footprint in most terrestrial communities, directly interacting with or indirectly affecting many other organisms. Ants exhibit a diverse array of behaviors, colony structures and genetic systems, the evolution and maintenance of which has intrigued evolutionary biologists (BOURKE & FRANKS 1995, CROZIER & PAMILO 1996). It is perhaps surprising, then, that our knowledge of ant phylogeny is quite incomplete, with morphological studies providing weak and sometimes contradictory resolution of the deeper branches of ant evolution (see below).

A recent landmark study by BOLTON (2003) reorganized the higher classification of ants, recognizing 21 extant (and 4 extinct) subfamilies. Numerous changes were also made at the tribal level. BOLTON's (2003) contribution represents a major advance in ant systematics because it breaks up non-monophyletic groups, such as Ponerinae

(sensu lato), into more defensible units that can be diagnosed with one or more putative autapomorphies. BOLTON (2003) did not carry out a phylogenetic analysis of the subfamilies (or tribes), but he clustered them into informal groups. In fact, the relationships among the ant subfamilies remain unclear. The lack of a consensus is evident from the discrepancies among the ant phylogenies presented by different authors (BROWN 1954, WILSON & al. 1967, TAYLOR 1978, HÖLDOBLER & WILSON 1990, BARONI URBANI & al. 1992, WARD 1994, GRIMALDI & al. 1997, GRIMALDI & AGOSTI 2000, GRIMALDI & ENGEL 2005, WILSON & HÖLDOBLER 2005). Where quantitative phylogenetic analyses have been carried out (BARONI URBANI & al. 1992, GRIMALDI & al. 1997) there is not strong support for most groups above the subfamily level.

Molecular phylogenetic studies have begun to contribute to the debate (SULLENDER & JOHNSON 1998, CHIOTIS & al. 2000, JOHNSON & al. 2003, WARD & BRADY 2003, BRADY 2003, ASTRUC & al. 2004, OHNISHI & al. 2004, SAUX & al. 2004, WARD & DOWNIE 2005) and show con-

siderable promise for disentangling the details of ant history. Most investigations to date have focused on a limited range of taxa, however, and/or sampled too few genes to provide good resolution.

Here we report on a collaborative attempt to resolve the ant Tree of Life, involving investigators from the University of California at Davis, the California Academy of Sciences, and the Smithsonian Institution. Supported by the US National Science Foundation AToL (Assembling the Tree of Life) program, the project entails comprehensive sampling of taxa and characters in order to obtain robust estimates of phylogenetic relationships. Specifically, the goals of the project are (1) to infer the phylogeny of the major ant lineages using a combination of morphological and molecular data; (2) to estimate the divergence times of these clades, employing molecular dating methods that incorporate rate heterogeneity and fossil data; and (3) to use the resulting phylogenetic and temporal framework to reconstruct key events in the history of ant evolution. The project also includes a significant bioinformatics component. Our findings will be organized and disseminated through the online portal AntWeb (www.antweb.org). The project runs for five years and is currently (2005) in its first year of operation.

It seems evident that molecular (DNA sequence) data will be most informative for recovering a well corroborated phylogeny of the ants, but morphological characters will also be incorporated and will assume particular importance for diagnosis of clades and the assignment of fossil taxa to these clades. The fossil data can in turn be used to constrain and inform the molecular dating analyses. With a well dated and robustly supported phylogeny we can more reliably reconstruct key events in the history of ant evolution.

Materials and methods

We are using BOLTON's (2003) classification as a framework for taxon sampling. We intend to sequence exemplar species from all the extant subfamilies (21) and tribes (63) of ants, and most of the 283 genera, for a total of about 400 terminal taxa. Outgroups will include a variety of other aculeate Hymenoptera, given uncertainty about the sister group of ants (BROTHERS 1999).

In this first year we have focused efforts on developing a set of molecular markers for phylogenetic inference. Currently this encompasses seven nuclear genes: 18S rDNA, 28S rDNA, abdominal-A, long wavelength rhodopsin, wingless, EF1-alpha F1, and EF1-alpha F2. Ultimately we will expand the molecular data set to include up to twenty genes. Primers used for the first five genes are based on those listed in WARD & DOWNIE (2005). Information on these and the EF1-alpha primers is available upon request from P.S. Ward or S.G. Brady. After excluding ambiguously aligned regions, these seven genes yield 5.8 kb of DNA sequence, of which ~ 1320 sites are parsimony-informative across our data set of ~ 100 ant taxa and three aculeate outgroups: *Apis* (Apidae), *Chyphotes* (Bradynobaenidae), and *Mischocyttarus* (Vespidae).

We are analyzing the data using standard parsimony and maximum likelihood procedures, as implemented in PAUP* 4.0b10 (SWOFFORD 2003). We are also conducting partitioned Bayesian analyses using MrBayes (HUELSENBECK & RONQUIST 2001, RONQUIST & HUELSENBECK

2003) version 3.1. These methods allow estimation not only of tree topology but also of branch lengths, which are critical for developing a timeframe of ant evolution. Fossil-calibrated molecular dating techniques (SANDERSON 2002, THORNE & KISHINO 2002) will be used to estimate divergence times for the major ant lineages.

Results

Preliminary findings include the following.

1) With respect to the three sampled outgroups – belonging to the families Apidae, Bradynobaenidae, and Vespidae – ants (Formicidae) form a monophyletic group. This is hardly a surprising result and it will be important to sample more widely among potential relatives of ants, including Scoliidae and Tiphiidae.

2) All ants except Leptanillinae, Agroecomyrmecinae, and most poneromorphs belong to a well supported clade (parsimony and likelihood bootstrap 100 %, Bayesian posterior probability 1.00) which we term the "formicoid clade" (Fig. 1). (The term is derived from the oldest available subfamily name within the clade – Formicinae – and should not be confused with the superfamily Formicoidea.) Evidence for this clade occurs in other recent molecular phylogenetic studies (WARD & BRADY 2003, OHNISHI & al. 2004, SAUX & al. 2004, WARD & DOWNIE 2005). Within the formicoids all the ant subfamilies recognized by BOLTON (2003) appear to be monophyletic, except possibly Cerapachyinae (see also BRADY & WARD in press).

3) Within the formicoid clade there is strong support for monophyly of myrmeciomorphs and dorylomorphs (sensu BOLTON 2003), but not for formicomorphs or myrmicomorphs. Poneromorph ants, with representatives both within and outside the formicoid clade, are an artificial group defined by a mixture of shared ancestral characters and convergent similarity.

4) Relationships among taxa outside the formicoid clade at the base of the ant tree (Agroecomyrmecinae, Amblyoponinae, Leptanillinae, Paraponerinae, Ponerinae, and Proceratiinae) are not well resolved with current data (Fig. 1). Our analyses indicate that the subfamily Leptanillinae is sister to all other ants, but this result might be an artifact of long-branch attraction (see BERGSTEN 2005).

Desiderata

Several uncommon taxa remain to be added to our molecular data set. We are particularly interested in obtaining fresh material (in 95 - 100 % EtOH) of the following ant genera: *Aenictogiton*, *Aneuretus*, *Anomalomyrma*, *Apomyrma*, *Aulacopone*, *Cheliomyrmex*, *Gesomyrmex*, *Lenomyrmex*, *Liomyrmex*, *Paratopula*, and *Stegomyrmex*. If readers of "Myrmecologische Nachrichten" have extra, alcohol-preserved specimens of any of these taxa we would be most grateful to receive samples. Material should be sent to the first author (P.S. Ward).

Concluding Remarks

A striking aspect of our findings is how well they accord with BOLTON's (2003) delineation of ant subfamilies. Bolton's schema was based solely on morphological characters, and the concordance is testimony to that author's keen insight. But the relationships among subfamilies revealed here by molecular data are novel and for the most part they were not anticipated by previous morphological stud-

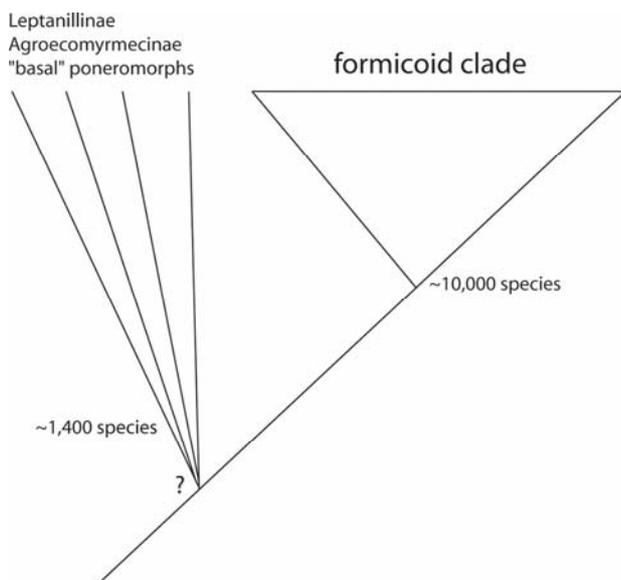


Fig. 1: Schematic diagram summarizing some of our preliminary findings on ant phylogeny. The formicoid clade includes the myrmeciomorphs, dorylomorphs, formicomorphs, Myrmicinae, Ectatomminae, and Heteroponerinae (sensu BOLTON 2003). The "basal" poneromorphs consist of Amblyoponinae, Paraponerinae, Ponerinae, and Proceratiinae. The polytomy indicates that most relationships among these latter taxa are not well resolved with current data, and it is likely that they do not form a monophyletic group. Species estimates refer to the total number of described extant species in each group.

ies. Some divergences between these groups evidently occurred close together in time and will be challenging to resolve. Multiple independent nuclear genes – perhaps as many as twenty or more – will probably be required to provide confident resolution of these "bushy" parts of the ant tree.

Our results suggest a pattern of sequential radiations – initial diversification of poneromorph-like lineages in the early Cretaceous 100 - 120 million years ago (along with the now extinct sphecomyrmines), followed by a more exuberant diversification of formicoids, starting in the middle to late Cretaceous and continuing into the Paleogene. These findings are broadly consistent with the historical scenario posited by WILSON & HÖLLDOBLER (2005), in which the later radiation of ants is associated with a switch from predation to omnivory and tending of angiosperm-feeding hemipterans. Our phylogenetic conclusions differ considerably from those presented in WILSON & HÖLLDOBLER (2005), however, and they highlight the fact that several ant groups considered ancient and "primitive", such as Myrmeciinae and some poneromorphs, in fact belong to the later originating formicoid clade. We emphasize, nevertheless, the provisional nature of our results and the need to sample additional taxa and characters before many aspects of the ant Tree of Life are brought to light. A more detailed analysis, with additional taxa and new molecular data, will be completed by the end of the year as a step toward achieving this goal.

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Zusammenfassung

Ameisen sind die weltweit dominante Gruppe eusozialer Organismen und nehmen eine zentrale ökologische Stellung in vielen terrestrischen Ökosystemen ein. Zum besseren Verständnis vieler Aspekte ihrer Ökologie und Evolution wird eine klare und stabile Phylogenie der Ameisen benötigt. Wir berichten über ein neues Projekt, das die Klärung der phylogenetischen Verwandtschaft der Großgruppen innerhalb der Ameisen zum Ziel hat. Dieses Projekt wird als Teil des AToL-Programms (Assembling the Tree of Life) von der US-amerikanischen National Science Foundation finanziert und hat eine Laufzeit von fünf Jahren. Die Durchführung des Projekts erfolgt in Zusammenarbeit von Wissenschaftlern der University of California in Davis, der California Academy of Sciences und der Smithsonian Institution. Im ersten Projektjahr (2004 - 2005) haben wir uns auf die Entwicklung eines multiplen Sets molekulargenetischer Marker und die Auswahl geeigneter Taxa konzentriert. Eine vorläufige Auswertung von Sequenzdaten (ca. 5800 bp) sieben nuklearer Gene von etwa 100 Arten erbrachte eine Reihe neuer Erkenntnisse, die einigen der früheren, auf morphologischen Untersuchungen basierenden, Befunden widersprechen. Während fast alle der 21 rezenten Subfamilien – entsprechend einer morphologisch begründeten Klassifizierung jüngerer Datums (BOLTON 2003) – monophyletisch erscheinen, gilt dies nur für zwei der insgesamt sechs Gruppen auf Supra-Subfamilien-Niveau (Dorylomorphe und Myrmeciomorphe). Starke Unterstützung (100 % Parsimonie- und Likelihood-Bootstrapping-Werte, 1.0 Bayesische Posterior-Wahrscheinlichkeit) findet sich für eine Gruppe, die wir hier den "formicoiden Zweig" nennen, und die alle rezenten Ameisen ausgenommen die Agroecomyrmecinae, Amblyoponinae, Leptanillinae, Paraponerinae, Ponerinae und Proceratiinae beinhaltet. Die Beziehungen zwischen den letzteren, früh divergierten (nicht-formicoiden) Ameisen sind nicht gut aufgelöst. Ein umfassender Bericht zum laufenden Projekt, unter Einbeziehung von Analysen weiterer Taxa und neuer molekulargenetischer Marker, ist für Jahresende zu erwarten.

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