

Inferring ant evolution in the age of molecular data (Hymenoptera: Formicidae)

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Abstract

Molecular data have revolutionized our understanding of ant evolution and systematics by making available large quantities of data and providing an independent character set to infer phylogenetic relationships. Although the first ant phylogeny from molecular sequence data was published less than 15 years ago, the field has grown rapidly with implications for all levels of ant biology. Not only has molecular data helped resolve the phylogenetic relationships of many ant groups, but with well-resolved phylogenies inferences about the evolution of morphology, ages of clades, diversification, associations with mutualists, behavior, development, and other areas of research in ant biology have benefited from this active avenue of research. Questions of speciation, population genetics, phylogeography, systematics, biogeography, and many other active lines of ant research are now routinely addressed with molecular data. With the ease of generating large amounts of sequence data increasing and costs decreasing the next frontier in myrmecology will be to insure that future ant biologists are able to take advantage of this important tool while still being trained in basic ant biology and taxonomy.

Key words: Formicidae, molecular systematics, phylogeny, phylogeography, DNA, diversification, ants, review.

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Introduction

Over the past few decades, evolutionary biologists have made substantial progress inferring the "Tree of Life". This increased understanding of the evolutionary relationships of the planet's organisms has come from several fields and to a great extent from value being placed on cataloguing, describing and understanding global biodiversity. Projects like the Tree of Life (www.tol.org) and the Encyclopedia of Life (www.eol.org) websites have helped promote and explain the importance of understanding the evolutionary history of the planet's biodiversity to the general public and scientific community. In many cases funding for this science has become available through national science organizations around the world. Scientists from a wide array of fields rely on the efforts of taxonomists and systematists to inform them if the organisms they are working on are closely related or even a single species, whether characters they are interested in have evolved independently, provide a timeline for the history of a particular species, and overall offer a framework for their findings in the larger evolutionary picture.

Not only has the ever improving technology of microscopes and image capture systems allowed us finer and clearer views of the morphological characters biologists have been looking at for centuries, but the use of molecular markers has become routine in the fields of phylogenetics, systematics, biogeography, development, behavior, ecology, phylogeography, and population biology. Theoretical advances in the field of phylogenetics and population genetics and increased computing power has also permitted better understanding of the evolutionary relation-

ships of fossil and modern taxa by providing the mechanisms to handle the analyses of large quantities of data.

Nowhere in ant research have we seen a more marked advance due to the use of molecular genetics than in the field of systematics / phylogenetics. From understanding how the extant ant subfamilies are related and the factors that shaped their diversification to unraveling species level relationships to using DNA "barcodes" to target areas and groups of understudied diversity, DNA has become a powerful tool for myrmecologists. CROZIER & al. (1995) published the first ant phylogeny based on DNA sequence data for the Australian jack-jumper ants from the genus *Myrmecia*. From this humble beginning with only 15 taxa sequenced for ~ 1150 base pairs (bp) of mitochondrial DNA, we have advanced in less than 15 years to a state where sequencing thousands of base pairs in an afternoon for hundreds of ant taxa is the norm. As of April 2009 there are over 13,600 individual nucleotide sequences for Formicidae available on the online GenBank database (www.ncbi.nlm.nih.gov) and plans to sequence the first six ant genomes have recently been announced (www.antgenomics.org). It should be noted that prior to DNA sequencing, many researchers were already taking advantage of allozyme (CROZIER & al. 1986, HILLIS 1987) and AFLP (KOOPMAN 2005, SIRVIO & al. 2006) data although these approaches will not be discussed in detail here. But with this ever-increasing mountain of DNA sequence data, what have we learned?

The use of molecular data in myrmecology has allowed researchers to infer phylogenetic relationships of ants from

higher level systematics through species level relationships with far more data than has traditionally been available with morphology. In addition, several myrmecologists have used molecular data to address questions of phylogeography and the utility of DNA for species identification and delimitation. With molecular phylogenies in hand (either generated by themselves or by previous authors), biologists have then been able to utilize this data to ask comparative questions of life history strategies and the evolution of morphology. One of the strengths that molecular data has provided is the ability to incorporate the fossil record to calibrate molecular clocks for dating events in the evolutionary history of the ants. Although there are potential problems and shortcomings of molecular data, the wealth of scientific opportunity this tool provides opens up many promising avenues of research for future myrmecologists. In addition with several ant genomes to become available in the near future, it is undoubtedly an exciting time to be involved in inferring ant evolution in the age of molecular data.

Phylogeny of the ants

Although there have been several significant contributions towards understanding the phylogenetic relationships of the subfamilies of Formicidae before the use of widespread molecular DNA sequencing (BROWN 1954, BARONI URBANI & al. 1992, BOLTON 1994, GRIMALDI & al. 1997, BOLTON 2003), one advantage is the relative independence of molecular characters compared with morphological characters (CROZIER 1983). In addition, the problem of limited character data led to a lack of resolution for many ant subfamily relationships and in some cases conflicting results between morphological studies. Although BROWN (1954) presented a phylogenetic tree for the subfamilies of Formicidae, this tree was not constructed from a morphological matrix, and from the words of the author "serves only to shake the peculiarly fixed faith with which some myrmecographers regard the speculations of [W.M.] Wheeler and his predecessors." BARONI URBANI & al. (1992) were the first to construct an extensive morphological matrix of 68 characters to infer the phylogenetic relationships of the ants. Building on the work of BARONI URBANI & al. (1992), GRIMALDI & al. (1997) included additional fossil taxa in their phylogenetic analysis. Even in these cases where over 60 morphological characters were scored for multiple species, phylogenetic relationships for several groups (i.e., Amblyoponinae; subfamily relationships) could not be resolved (BARONI URBANI & al. 1992, GRIMALDI & al. 1997). These important morphological studies provided a framework to understand the evolution of the Formicidae and hypotheses on the evolution of the ants to test not only with additional morphological studies, but also using molecular sequence data. One of the strengths that these studies provide that still overshadows all molecular phylogenetic studies is their ability to include extinct taxa to infer their phylogenetic affinities. With this said, all fossil information is not lost in modern molecular phylogenetics as data on the minimum age for modern groups is frequently used to date events in molecular phylogenies (see fossils and molecular clock section below).

Several early attempts using molecular sequence data from limited sampling were made to understand the higher-level phylogenetic relationships of the ants (15 species:

OHNISHI & al. 2003, 49 species: ASTRUC & al. 2004). Although these two studies provided important contributions to the field of ant systematics, the first major hurdles in resolving the major relationships of the ant subfamilies were overcome with the publications of MOREAU & al. (April 2006: 143 species from 139 genera) and BRADY & al. (November 2006: 162 species from 125 genera).

MOREAU & al. (2006) presented the first large-scale molecular phylogeny of the ants with 19 of the then known 20 modern subfamilies represented. This study recovered the monophyly of all the included subfamilies with the exception of the Cerapachyinae based on the subfamily diagnosis of BOLTON (2003). Support of the monophyly of Amblyoponinae was lacking, although members of this subfamily included in the study did group together topologically and had received support for their monophyly in a previous molecular phylogenetic study (SAUX & al. 2004). The finding of Leptanillinae as sister to all other modern ants is striking and had never been suggested based on morphology. Leptanilline ants are minute and due to their subterranean lifestyle are pale in color and eyeless, hardly how previous authors had hypothesized the earliest living ant lineage would appear (WHEELER 1910, BROWN 1954, WILSON & al. 1967, WILSON 1971, WARD 1994). This finding of the Leptanillinae as a possible early lineage in a molecular phylogenetic study was not entirely novel as this relationship was suggested in a previous study on a much more restrictive set of taxa (SAUX & al. 2004), and considered as potentially controversial by a subsequent study (BRADY & al. 2006). Although the more recent findings by RABELING & al. (2008) suggest this finding of Leptanillinae as an early lineage may reflect the true evolutionary history of the Formicidae. In the study of MOREAU & al. (2006), inclusion of the rich fossil record of ants allowed for molecular clock analyses that provided a timeline for the evolution of the ants and suggested that the ants began diversifying much earlier than had been previously suggested from the fossil record. From this evolutionary timeline, MOREAU & al. (2006) found a correlation with the rapid diversification of the ants with that of the flowering plants and many sap-feeding insects, a pattern that had been anticipated by WILSON & HÖLLDOBLER (2005).

In the same year, BRADY & al. (2006) published the next large molecular phylogeny of the ants including all 20 of the then known ant subfamilies. Overall the findings of BRADY & al. (2006) were in agreement with those of MOREAU & al. (2006) and as stated by CROZIER (2006), "There is now the emergence of the promise of stability in ant phylogeny." One important difference between the two studies was the question of the effect of alternative rootings on the topology. To address the potential problem of the long branch from the ants to any other Hymenoptera outgroups, BRADY & al. (2006) experimented with changing the root position on the unrooted ant phylogeny and the effect this had on reconstructing the earliest lineages within the Formicidae. From their results, they concluded that several other possible rootings could not be statistically rejected suggesting that Leptanillinae may not be the sister to modern ants as found in MOREAU & al. (2006).

More recently using DNA from three legs of the single known specimen of a new species and subfamily from Brazil, RABELING and colleagues (2008) were able to demonstrate this new species may present one of the earliest



Fig. 1: Phylogeny of the extant Formicidae based on preliminary results (C.S. Moreau & C.D. Bell, unpubl.) from the combined molecular data of MOREAU & al. (2006), BRADY & al. (2006), and RABELING & al. (2008). Outgroup taxa not shown. Branch lengths, which end at the right edge of the triangles, are proportional to estimated divergence. Height of subfamily triangles are proportional to number of taxa included in molecular analysis and do not reflect the number of known species in each clade. First branching event within a subfamily clade is denoted by the origin of the triangles and does not necessarily reflect the origin of the diversification of the modern lineage. All subfamilies were recovered as monophyletic, with the exception of Cerapachyinae (indicated by bracket). Included are estimates of number of extant species for each of the subfamilies following the subfamily names after BOLTON & al. (2007).

living groups of ants. Their finding of Martialinae as sister to all remaining extant ants also helped confirm the placement of Leptanillinae as an early lineage within the modern ants. Surprisingly this putative sister lineage to modern ants shared some startling features with leptanillines, the other

hypothesized earliest lineage that had been suggested based on molecular data, as both are essentially eyeless, pale, and likely have an entirely subterranean lifestyle. Although the sister position of Martialinae and Leptanillinae to the modern ants seems counterintuitive based on morphology and

ecology, this finding suggests that the earliest ants may have been able to endure the test of evolutionary time by adopting a subterranean lifestyle.

Preliminary results are presented here for a study combining the molecular sequence data of MOREAU & al. (2006), BRADY & al. (2006), and RABELING & al. (2008) into a single large dataset (C.S. Moreau & C.D. Bell, unpubl.) to infer the phylogenetic relationships of the living ants and tease apart the timeframe involved in the diversification of this ecologically important and abundant group (Fig. 1). Based on these preliminary results the two earliest lineages Martialinae and Leptanillinae are recovered as a basal polytomy, but their sister relationship to all other modern ants is recovered as in MOREAU & al. (2006) and RABELING & al. (2008). In addition, another interesting finding is the two sister subfamilies Ectatomminae and Heteroponerinae are recovered as sister to the Myrmicinae, a finding that had been suggested by BROWN (1958) in his statement that the "subfamily Myrmicinae arose from an ectatommine ancestor."

Although these molecular phylogenies have helped provide a stable framework for the relationships of the ant subfamilies, one of the most striking results is how accurately BOLTON (1994, 2003) was able to circumscribe the genera within each subfamily (WARD & al. 2005). The contributions of BOLTON (1994, 2003) are all the more remarkable when one considers that he was able to delimit each subfamily based on intuitive insights and familiarity with morphological characters and Bolton's contributions extend well beyond higher level ant systematics (BOLTON 1990a, 1990b, BOLTON 1995, BOLTON 1999, BOLTON 2007, among others).

Molecular systematics above the genus level

Much has been gained in ant systematics from the use of molecular sequence data beyond a better understanding of the higher-level relationships. DNA sequencing has provided a rapid and useful way to understand relationships among members of subfamilies, tribes, or genera and an independent means to test morphological hypotheses. To demonstrate the contributions that molecular data has afforded ant systematics above the genus level, I will outline the findings of several studies here. The first attempt to understand the phylogenetic relationships of the fungus-growing ants was published in 1998 by WETTERER & al. with a much more complete study completed recently by SCHULTZ & BRADY (2008) affording a clearer understanding of the evolution of ant agriculture. CHIOTIS & al. (2000) found for the Dolichoderinae the relationships recovered using sequence data were in agreement for the most part with morphological studies, a finding that most molecular studies on ant systematics also find. For the army ants, BRADY (2003) was able to demonstrate that the army ant syndrome, which Brady outlines as a suite of behavioral and reproductive traits, evolved once in the evolutionary history of the group. This finding was important and demonstrated that army ants shared a common ancestor and had not evolved this syndrome independently through convergence on separate continents. Also in 2003 two additional studies were published addressing the phylogenetic relationships of the Formicinae (JOHNSON & al. 2003) and the Myrmeciinae (WARD & BRADY 2003). In using sequence data to examine the relationships of members of the Amblyoponinae, SAUX & al. (2004) were able to couple the find-

ings from their molecular data with morphology to provide a morphological diagnosis of the subfamily, which included the inclusion of *Apomyrma* within Amblyoponinae. Using a combination of morphological and molecular data, WARD & DOWNIE (2005) were able to infer the phylogenetic relationships among the species and infer that members of Pseudomyrmecinae have evolved obligate associations with domatia-bearing ant-plants at least twelve times. OUELLETTE & al. (2006) investigated the relationships among the poneroid subfamilies using molecular sequence data to look across several subfamilies. All these studies highlight that using molecular sequence data have permitted us to begin to understand the complex and intriguing evolutionary history of ants above the genus level and provide an independent means to test questions of morphological, behavioral, and ecological evolution. In almost all cases the morphological characters that allowed early myrmecologists to group these lineage into single taxonomic units have been confirmed with molecular data and these studies have highlighted the need for additional taxonomic work within many of the groups explored.

Species level phylogenies

At an even finer scale, many ant biologists have used molecular systematics to help address questions of species level relationships. From the first ant molecular phylogeny of CROZIER & al. (1995) the number of publications on ant phylogenetic relationships using molecular sequence data has grown at an astonishing rate allowing for the investigation of the species level relationships for a large number of genera. The studies included here are far from exhaustive, but highlight some of the ways that ant researchers have been able to use the findings of molecular data to gain insights into the evolution of the ants. AYALA & al. (1996) were able to infer multiple independent colonizations of *Cecropia* trees by *Azteca* ants. Other notable molecular phylogenies for ant genera include *Acropyga* (LAPOLLA & al. 2006), *Myrmecocystus* (KRONAUER & al. 2004), *Lasius* (JANDA & al. 2004), *Pseudomyrmex* and *Tetraoponera* (WARD & DOWNIE 2005), *Myrmecia* (HASEGAWA & CROZIER 2006), *Myrmecina* (STEINER & al. 2006a), *Dorylus* (KRONAUER & al. 2007), *Trachymyrmex* (RABELING & al. 2007), *Pheidole* (MOREAU 2008), *Atta* (SOLOMON & al. 2008), *Lasius* (MARUYAMA & al. 2008), and *Linepithema* (WILD 2009). One major finding that has resulted from all of these studies is there is always more work to be done, whether it is including missing taxa, finding additional molecular markers, or looking for morphological or other characters that may support the findings of the molecular data when they are in conflict. For every conclusion that is inferred from these molecular phylogenies, many more questions are uncovered and others left unresolved.

Phylogeography

Modern molecular methods have also advanced the field of phylogeography permitting us to better understand the historical processes that shape distributions of modern species (AVISE 2009). Inferring phylogeographic patterns within ant lineages has improved our knowledge of how factors including population expansion, population bottlenecks, vicariance and migration have influenced ant populations found today. Researchers have been able to document how ants and their mutualistic partners have shaped

the distribution and diversification of each other and the role that historical climate change has played in the understanding the genetic structure of modern populations (QUEK & al. 2007, MIKHEYEV & al. 2008). Phylogeography studies can also examine whether past distributions of continents, islands, and seas during glacial and other periods explain current genetic diversity as was found in weaver ants (AZUMA & al. 2006), although SCHLÜNS & al. (2009) found in the same species that phylogeny does not predict social structure. In some cases the amount of genetic structure found within ant populations has led researchers to the conclusion that recent speciation explains the data (GOROPASHNAYA & al. 2004a). While at the other extreme a lack of genetic structure among what were considered separate species or populations suggest that much more gene flow is occurring than had been previously suspected or that potentially selection on morphology as well as gene flow explains the data (GOROPASHNAYA & al. 2004b, STEINER & al. 2006b). Population genetic information has also provided an avenue of research to understand alternative social strategies including differing queen size and queen number in separate populations (SCHLICK-STEINER & al. 2007) and the genetic structure of a parasitic ant and its host ant (BEIBL & al. 2007). Another productive line of phylogeographic research has been to examine the genetic structure between native and introduced populations of ants (TSUTSUI & CASE 2001, AHRENS & al. 2005, MIKHEYEV & MUELLER 2007), with the hope that these findings may provide a better understanding of the factors that promote the ecological success of invasive species outside of their native ranges. In some cases inferences from the phylogeography of ants may explain broader patterns of diversity (SOLOMON & al. 2008). Overall understanding the factors shaping ant populations will not only help us to better understand the evolutionary history of the Formicidae, but may also allow us to explain broader patterns in biology.

DNA species identification and delimitation

The use of DNA alone to inform species identifications and delimit species boundaries has not been well tested in ants (SEIFERT 2009) and remains a controversial idea across almost all of life outside of bacteria (MORITZ & CICERO 2004). Determining the criterion for delimiting species is not always clear, although attempts have been made to include DNA as a measure (SITES & MARSHALL 2004). Although the use of a DNA "barcode" (a small region of DNA) to document and understand diversity has many potential pitfalls including unknowingly amplifying pseudo genes, issues caused by hybridization / introgression, among others (RUBINOFF & al. 2006), this tool can be very useful in ant biology. SMITH & al. (2005) were able to demonstrate that in the Malagasy region the use of the DNA "barcode" was effective in assessing biodiversity of ant communities. More recently FISHER & SMITH (2008) have used the information from DNA "barcoding" coupled with careful morphological taxonomy to revise two genera of ants in Madagascar. The potential for this molecular tool in ant biology is growing (CROZIER & al. in press), but additional studies of how comparable the results are from DNA "barcodes" across ants and other species are necessary to infer their widespread utility (MORITZ & CICERO 2004). Another approach is to couple information gained from DNA with other sources of information (i.e., morphology, behavior, ecology, etc.) and

to use all of these resources combined to guide the identification of species and delimit species boundaries (RUBINOFF & HOLLAND 2005).

Uses of ant phylogenies

Although the taxonomic information provided by phylogenies is worthwhile in and of itself, one interesting byproduct is how the phylogenies are eventually used to understand other traits, behaviors, and characters of interest. In some cases these questions are addressed by the authors of the original phylogenetic study, while in other cases researchers interested in a specific question have used the phylogeny generated in previous studies to gain insight into the evolutionary history of their question of interest. With a phylogeny for a group available understanding how a trait has evolved within a lineage is put within an evolutionary framework and phylogenetic uncertainty can be taken into account in comparative analyses such as the reconstruction of ancestral states and the evolutionary association of states across characters (RONQUIST 2004). Using these methods, we have begun to understand the evolution of a diversity of traits, behaviors, and characters in ants including genome size (TSUTSUI & al. 2008), trophobiosis with Hemiptera (OLIVER & al. 2008), territoriality and predatory behavior (DEJEAN & al. 2007), body size and ant-plant mutualisms (CHAMBERLAIN & HOLLAND 2008), reproductive division of labor (KHILA & ABOUHEIF 2008), associations with endosymbiotic bacteria (DEGNAN & al. 2004, RUSSELL & al. 2009), mutualism specialization of plant-ants (KAUTZ & al. in press), and morphological, behavioral and developmental trait mapping (JOHNSON & al. 2003, MOREAU 2008, SPAGNA & al. 2008). What these and the countless other studies that make use of the information provided by a phylogeny demonstrate is in order to understand the evolution of any character or trait, one must first have an understanding of the evolutionary history of the group of interest. Without this information (the phylogeny) inferences about homology or development of a trait are only speculations.

Fossils and molecular clocks

The notion of a molecular clock is far from a new concept (ZUCKERKANDL & PAULING 1962), but with the recent ability to sequence large amounts of DNA inexpensively and quickly, the use of molecular clock methods have become widely used in evolutionary biology (PULQUERIO & NICHOLS 2007). Molecular clock methods allow the user to calibrate the rate of molecular evolution expected within the data from fossils, biogeographic data or knowledge of the rates of molecular change within a specific gene or lineage. With an incredibly rich fossil record, widespread distributions, and more molecular genetic material becoming available at an ever-increasing rate, the ants are the ideal group to take advantage of molecular clock methods.

The first study to incorporate molecular clock methods to attempt to date the extant ants with sequence data was that of CROZIER & al. (1997). Using sequence divergence between mitochondrial DNA sequences from six ant subfamilies and a vespid wasp, CROZIER & al. (1997) inferred a Jurassic origin for the ants. This finding was the first molecular clock study of ant evolution to push back the age of the group well beyond the oldest known fossils. One very interesting implication of this study, highlighted by the

authors is that this finding suggests ants arose before the breakup of Pangaea and this may account for the current distributions of many of the major ant groups. Interestingly a more recent study has supported these findings using far more taxa and incorporating fossil information (MOREAU & al. 2006).

Several studies have used fossil information to put a timeline on the evolution of specific lineages within the modern ants. BRADY (2003) was able to demonstrate that army ants have their origin in the mid-Cretaceous consistent with a Gondwanan origin. This finding of an age of ~ 105 million years (Ma) for the army ants reinforces the hypothesis of a single origin of the group with subsequent divergence after the breakup of Gondwana into Africa and South America. To understand the timeline for the evolution of the subfamily Myrmeciinae, currently found only in the Australian region, WARD & BRADY (2003) incorporated molecular data with seven fossil minimum calibration points. Interestingly the fossils of Myrmeciinae are all found outside of the Australian region, demonstrating that at some time in the ~ 74 million years of evolutionary history of this group, they were once far more widespread. In an attempt to understand the diversification of a "hyperdiverse" ant lineage, MOREAU (2008) used molecular clock methods to estimate an age for the genus *Pheidole* and the number of speciation events per million years. From this study, MOREAU (2008) found that the genus *Pheidole* has undergone 0.120 - 0.114 (incorrectly underestimated as 0.108 - 0.103 in the original publication due to an inadvertent mathematical error) speciation / diversification events per million years, a rate higher than found in many groups in which this has been investigated.

Resolving the timing for the origin and diversification for the modern (extant) ants has become a topic of interest in the ant phylogenetics community. The oldest known formicid fossils date back to the Albian (~ 100 million years) (GRIMALDI & al. 2002, PERRICHOT & al. 2007a, 2007b) leading to the question of how long before this did ants begin to diversify? MOREAU & al. (2006) found an age of 140 - 168 million years for the crown group ants with a burst in diversification, which seems to be correlated with the rise of the flowering plants (angiosperms). Using different calibration points, BRADY & al. (2006) found a younger age for the ants (115 - 135 Ma), which is more in line with the fossil evidence, although still not entirely supported. Although we are still not certain of the age of the modern crown group ants, I am hopeful that with the ever-increasing molecular data available, increased taxon sampling, and incorporation of as many fossil calibration points as possible, we will be able to infer when this diverse group originated.

Potential problems

No method is without its shortcomings and molecular tools are no exception. Some shortcomings of molecular data are shared among all types of data. These include missing taxa in the analyses and data that are unable to resolve the particular question addressed. On the other hand, molecular data provide an independent means to address questions of morphological evolution, which is difficult to do otherwise. Although molecular data are susceptible to unique issues such as amplification of pseudogenes (numts and others), introgression, saturation and long branch attraction (FELSENSTEIN 1978, BROWER & al. 1996, SONG & al. 2008), the ease at

which you can gather immense amounts of data make them often the data source of choice. Another important and interesting result of the recent interest in molecular sequence data from ants is the discrepancies between the fossil and molecular clock estimates. Although it is nearly impossible to determine which, if either, is correct hopefully with the incorporation of more and more sequence data and well-placed fossil calibration points, we may be able to come to a common ground between the two sources of data. Overall, molecular data provides a source of information that has allowed us to advance the state of ant biology in ways that would otherwise be difficult or impossible.

Conclusions

Without a doubt the use of molecular systematics has revolutionized our understanding of ant evolution. Molecular sequence data has allowed us to begin to unravel even some of the more difficult to infer phylogenetic relationships within the ants from the higher-level relationships (Fig. 1) to species and population level dynamics. Not only has systematics benefited from this tool, but also areas such as evolutionary development, behavior, ecology, and other disciplines have incorporated the findings from molecular phylogenetics to place their areas of research in an evolutionary framework.

The ant fossil record is not entirely in agreement with molecular clock estimates, which suggest older ages than the fossil record has provided and this is not unique to the ants. Is this due to an incomplete fossil record with several ghost lineages still to be found or are the molecular clock estimates simply far too old? As we are able to gather additional molecular data from a greater number of species, subject these data to newer and better molecular clock methods, and as additional fossils are uncovered, it will be interesting to try to grapple with these discrepancies between the two sources of data.

One of the major hurdles we are on the cusp of overcoming to understand the evolutionary history of the ants is that several ant genomes will finally be sequenced (www.antgenomics.org). Although we now have six ant genomes from three separate subfamilies in the genome sequencing queue, increasing the taxonomic diversity of genomes sequenced is as important as looking at genomic differences among closely related ant species. Many interesting questions can be addressed from the sequencing of ant genomes, including understanding factors that may be shaping the molecular evolution of the genome and how and why genomes between ant species differ. In addition to these direct questions, there are many indirect benefits that await myrmecologists. The availability of ant genomes will facilitate the development of additional molecular markers for phylogenetic and developmental studies from the higher levels to population genetics. Furthermore, we will begin to identify regions of the genome that have undergone selection among different ant lineages and between ants and other hymenopterans, which may provide insight to the genomic changes associated with the origin of eusociality. This potential outcome of genomic data is one of the most exciting.

Although ants provide a novel system to address questions of levels of eusociality, caste development and behavior, among others, one factor may potentially always keep ants from becoming a laboratory model system. Since most

ant species require a mating flight for reproduction, manipulating them in the laboratory and creating controlled crosses is difficult (but see, e.g., CUPP & al. 1973, HUNG 1973, BUSCHINGER 1975, BALL & al. 1983, WINTER & BUSCHINGER 1986). Unless we are able to find a broad number of species that will mate easily in the laboratory or perfect the methods to inseminate ant queens in the laboratory, we will not be able to see this biologically interesting group realized as a true model system.

Lastly as molecular sequencing becomes easier and cheaper the next frontier in ant biology will be to find myrmecologists that are able to couple molecular data with knowledge of ant taxonomy and natural history to tease apart the complex evolutionary biology of the ants. Without a clear understanding of what you are sequencing and some knowledge of the basic biology of the species little is gained by having its DNA sequence alone. In closing to borrow (and modify) a quote from Theodosius DOBZHANSKY (1973): "Nothing in [ant] biology makes sense except in the light of [phylogenetics and] evolution."

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Zusammenfassung

Molekulare Daten haben unser Verständnis von Evolution und Systematik der Ameisen revolutioniert, indem sie unabhängige Merkmale für die Rekonstruktion phylogenetischer Beziehungen zugänglich gemacht und eine wesentliche Steigerung des Datenumfangs ermöglicht haben. Die erste Ameisenphylogenie auf der Basis molekularer Daten wurde erst vor weniger als 15 Jahren veröffentlicht, aber die Forschungsrichtung ist seither rasch gewachsen und hat die biologische Forschung über Ameisen auf allen Ebenen verändert. Nicht nur haben molekulare Daten geholfen, die phylogenetischen Beziehungen vieler Ameisengruppen zu klären, sondern durch gut unterstützte Phylogenien war es auch möglich, weiterreichende Schlüsse zur Evolution der Morphologie, zum Alter von Gruppen, zu Diversifizierung, Beziehungen mit Mutualisten, Verhalten, Entwicklungsbiologie und anderen Sparten der Ameisenbiologie zu ziehen. So werden heute Fragen zu Speziation, Populationsgenetik, Phylogeographie, Systematik, Biogeographie und vielen anderen Bereichen in der myrmekologischen Forschung

routinemäßig mit molekularen Daten bearbeitet. Es wird bei weiter sinkenden Kosten immer einfacher werden, große Sequenzdatensätze zu generieren. Die nächste Herausforderung für die Myrmekologie wird sein, einerseits sicherzustellen, dass zukünftige Ameisenforscher diese wichtigen Werkzeuge einsetzen können, und andererseits, dass sie auch weiterhin eine gute Ausbildung in der grundlegenden Biologie und der Taxonomie der Ameisen erfahren.

References

- AHRENS, M.E., ROSS, K.G. & SHOEMAKER, D.D. 2005: Phylogeographic structure of the fire ant *Solenopsis invicta* in its native South American range: Roles of natural barriers and habitat connectivity. – *Evolution* 59: 1733-1743.
- ASTRUC, C., JULIEN, J.F., ERRARD, C. & LENOIR, A. 2004: Phylogeny of ants (Formicidae) based on morphology and DNA sequence data. – *Molecular Phylogenetics and Evolution* 31: 880-893.
- AVISE, J.C. 2009: Phylogeography: retrospect and prospect. – *Journal of Biogeography* 36: 3-15.
- AYALA, F.J., WETTERER, J.K., LONGINO, J.T. & HARTL, D.L. 1996: Molecular phylogeny of *Azteca* ants (Hymenoptera: Formicidae) and the colonization of *Cecropia* trees. – *Molecular Phylogenetics and Evolution* 5: 423-428.
- AZUMA, N., OGATA, K., KIKUCHI, T. & HIGASHI, S. 2006: Phylogeography of Asian weaver ants, *Oecophylla smaragdina*. – *Ecological Research* 21: 126-136.
- BALL, D.E., MIRENDA, J.T., SORENSEN, A.A. & VINSON, S.B. 1983: Instrumental insemination of the fire ant, *Solenopsis invicta*. – *Entomologia Experimentalis et Applicata* 33: 195-202.
- BARONI URBANI, C., BOLTON, B. & WARD, P.S. 1992: The internal phylogeny of ants (Hymenoptera, Formicidae). – *Systematic Entomology* 17: 301-329.
- BEIBL, J., BUSCHINGER, A., FOITZIK, S. & HEINZE, J. 2007: Phylogeny and phylogeography of the Mediterranean species of the parasitic ant genus *Chalepoxenus* and its *Temnothorax* hosts. – *Insectes Sociaux* 54: 189-199.
- BOLTON, B. 1990a: The higher classification of the ant subfamily Leptanillinae (Hymenoptera, Formicidae). – *Systematic Entomology* 15: 267-282.
- BOLTON, B. 1990b: Army ants reassessed – the phylogeny and classification of the doryline section (Hymenoptera, Formicidae). – *Journal of Natural History* 24: 1339-1364.
- BOLTON, B. 1994: Identification guide to the ant genera of the world. – Harvard University Press, Cambridge, MA, 222 pp.
- BOLTON, B. 1995: A new general catalogue of the ants of the world. – Harvard University Press, Cambridge, MA, 504 pp.
- BOLTON, B. 1999: Ant genera of the tribe Dacetonini (Hymenoptera: Formicidae). – *Journal of Natural History* 33: 1639-1689.
- BOLTON, B. 2003: Synopsis and classification of Formicidae. – *Memoirs of the American Entomological Institute* 71: 1-370.
- BOLTON, B. 2007: Taxonomy of the dolichoderine ant genus *Techonormyx* MAYR (Hymenoptera: Formicidae) based on the worker caste. – *Contributions of the American Entomological Institute* 35: 1-149.
- BOLTON, B., ALPERT, G.D., WARD, P.S. & NASKRECKI, P. 2007: Bolton's catalogue of ants of the world. – Harvard University Press, Cambridge, MA, CD-ROM.
- BRADY, S.G. 2003: Evolution of the army ant syndrome: the origin and long-term evolutionary stasis of a complex of behavioral and reproductive adaptations. – *Proceedings of the National Academy of Sciences of the United States of America* 100: 6575-6579.

- BRADY, S.G., SCHULTZ, T.R., FISHER, B.L. & WARD, P.S. 2006: Evaluating alternative hypotheses for the early evolution and diversification of ants. – Proceedings of the National Academy of Sciences of the United States of America 103: 18172-18177.
- BROWER, A.V.Z., DESALLE, R. & VOGLER, A. 1996: Gene trees, species trees, and systematics: a cladistic perspective. – Annual Review of Ecology and Systematics 27: 423-450.
- BROWN, W.L. JR. 1954: Remarks on the internal phylogeny and subfamily classification of the family Formicidae. – Insectes Sociaux 1: 21-31.
- BROWN, W.L. JR. 1958: Contributions toward a reclassification of the Formicidae. II. Tribe Ectatommini (Hymenoptera). – Bulletin of the Museum of Comparative Zoology 118: 173-362.
- BUSCHINGER, A. 1975: Eine genetische Komponente im Polymorphismus der dulotischen Ameise *Harpagoxenus sublaevis*. – Naturwissenschaften 62: 239-240.
- CHAMBERLAIN, S.A. & HOLLAND, J.N. 2008: Density-mediated, context-dependent consumer resource interactions between ants and extrafloral nectar plants. – Ecology 89: 1364-1374.
- CHIOTIS, M., JERMIIN, L.S. & CROZIER, R.H. 2000: A molecular framework for the phylogeny of the ant subfamily Dolichoderinae. – Molecular Phylogenetics and Evolution 17: 108-116.
- CROZIER, R.H. 1983: Genetics and insect systematics: retrospect and prospect. In: HIGHLEY, E. & TAYLOR, R.W. (Eds.): Australian systematic entomology: a bicentenary perspective. – Commonwealth Scientific and Industrial Research Organization, Melbourne, pp. 80-92.
- CROZIER, R.H. 2006: Charting uncertainty about ant origins. – Proceedings of the National Academy of Sciences of the United States of America 103: 18029-18030.
- CROZIER, R.H., AGAPOW, P.-M. & SMITH, M.A. in press: Conservation genetics: from species to habitats. – Biology International.
- CROZIER, R.H., DOBRIC, N., IMAI H.T., GRAUR, D., CORNUET, J.M. & TAYLOR, R.W. 1995: Mitochondrial-DNA sequence evidence on the phylogeny of Australian jack-jumper ants of the *Myrmeda pilosula* complex. – Molecular Phylogenetics and Evolution 4: 20-30.
- CROZIER, R.H., JERMIIN, L.S. & CHIOTIS, M. 1997: Molecular evidence for a Jurassic origin of ants. – Naturwissenschaften 84: 22-23.
- CROZIER, R.H., PAMILO, P., TAYLOR, R.W. & CROZIER, Y.C. 1986: Evolutionary patterns in some putative Australian species in the ant genus *Rhytidoponera*. – Australian Journal of Zoology 34: 535-560.
- CUPP, E.W., O'NEAL, J., KEARNEY, G. & MARKIN, G.P. 1973: Forced copulation of imported fire ant reproductives. – Annals of the Entomological Society of America 66: 743-745.
- DEGNAN, P.H., LAZARUS, A.B., BROCK, C.D. & WERNEGREEN, J.J. 2004: Host-symbiont stability and fast evolutionary rates in an ant-bacterium association: cospeciation of *Camponotus* species and their endosymbionts, *Candidatus Blochmannia*. – Systematic Biology 53: 95-110.
- DEJEAN, A., CORBARA, B., LEPONCE, M. & ORIVEL, J. 2007: Ants of rainforest canopies: the implications of territoriality and predatory behavior. – Functional Ecosystems and Communities 1: 105-120.
- DOBZHANSKY, T. 1973: Nothing in biology makes sense except in the light of evolution. – The American Biology Teacher 35: 125-129.
- FELSENSTEIN, J. 1978: Cases in which parsimony or compatibility methods will be positively misleading. – Systematic Zoology 27: 401-410.
- FISHER, B.L. & SMITH, M.A. 2008: A revision of Malagasy species of *Anochetus* MAYR and *Odontomachus* LATREILLE (Hymenoptera: Formicidae). – Public Library of Science One 3: 1-23.
- GOROPASHNAYA, A.V., FEDOROV, V.B. & PAMILO, P. 2004a: Recent speciation in the *Formica rufa* group ants (Hymenoptera, Formicidae): inference from mitochondrial DNA phylogeny. – Molecular Phylogenetics and Evolution 32: 198-206.
- GOROPASHNAYA, A.V., FEDOROV, V.B., SEIFERT, B. & PAMILO, P. 2004b: Limited phylogeographical structure across Eurasia in two red wood ant species *Formica pratensis* and *F. lugubris* (Hymenoptera, Formicidae). – Molecular Ecology 13: 1849-1858.
- GRIMALDI, D., AGOSTI, D. & CARPENTER, J.M. 1997: New and rediscovered primitive ants (Hymenoptera: Formicidae) in Cretaceous amber from New Jersey, and their phylogenetic relationships. – American Museum Novitates 3208: 1-43.
- GRIMALDI, D.A., ENGEL, M.S. & NASCIMBENE, P.C. 2002: Fossiliferous Cretaceous amber from Myanmar (Burma): its rediscovery, biotic diversity, and paleontological significance. – American Museum Novitates 3361: 1-71.
- HASEGAWA, E. & CROZIER, R.H. 2006: Phylogenetic relationships among species groups of the ant genus *Myrmecia*. – Molecular Phylogenetics and Evolution 38: 575-582.
- HILLIS, D.M. 1987: Molecular versus morphological approaches to systematics. – Annual Review of Ecology and Systematics 18: 23-42.
- HUNG, A.C.F. 1973: Inducing mating in *Formica* ants (Hymenoptera: Formicidae). – Entomological News 84: 310-313.
- JANDA, M., FOLKOVA, D. & ZRZAVY, J. 2004: Phylogeny of *Lasius* ants based on mitochondrial DNA and morphology, and the evolution of social parasitism in the Lasini (Hymenoptera: Formicidae). – Molecular Phylogenetics and Evolution 33: 595-614.
- JOHNSON, R.N., AGAPOW, P.M. & CROZIER, R.H. 2003: A tree island approach to inferring phylogeny in the ant subfamily Formicinae, with especial reference to the evolution of weaving. – Molecular Phylogenetics and Evolution 29: 317-330.
- KAUTZ, S., LUMBSCH, H.T., WARD, P.S. & HEIL, M. in press: How to prevent cheating: a digestive specialization ties mutualistic plant-ants to their ant-plant partners. – Evolution.
- KHILA, A. & ABOUHEIF, E. 2008: Reproductive constraint is a developmental mechanism that maintains social harmony in advanced ant societies. – Proceedings of the National Academy of Sciences of the United States of America 105: 17884-17889.
- KOOPMAN, W.J.M. 2005: Phylogenetic signal in AFLP data sets. – Systematic Biology 54: 197-217.
- KRONAUER, D.J.C., HÖLLDOBLER, B. & GADAU, J. 2004: Phylogenetics of the new world honey ants (genus *Myrmecocystus*) estimated from mitochondrial DNA sequences. – Molecular Phylogenetics and Evolution 32: 416-421.
- KRONAUER, D.J.C., SCHÖNING, C., VILHELMSSEN, L.B. & BOOMSMA, J.J. 2007: A molecular phylogeny of *Dorylus* army ants provides evidence for multiple evolutionary transitions in foraging niche. – BioMed Central Evolutionary Biology 7: e56.
- LAPOLLA, J.S., KJER, K.M., SCHULTZ, T.R. & BISCHOFF, J.F. 2006: Phylogenetic position of the ant genus *Acropyga* ROGER (Hymenoptera: Formicidae) and the evolution of trophophoresy. – Insect Systematics and Evolution 37: 197-212.
- MARUYAMA, M., STEINER, F.M., STAUFFER, C., AKINO, T., CROZIER, R.H. & SCHLICK-STEINER, B.C. 2008: A DNA and morphology based phylogenetic framework of the ant genus *Lasius* with hypotheses for the evolution of social parasitism and fungiculture. – BioMed Central Evolutionary Biology 8: e237.
- MIKHEYEV, A.S. & MUELLER, U.G. 2007: Genetic relationships between native and introduced populations of the little fire ant

- Wasmannia auropunctata*. – Diversity and Distributions 13: 573-579.
- MIKHEYEV, A.S., VO, T. & MUELLER, U.G. 2008: Phylogeography of post-Pleistocene population expansion in a fungus-gardening ant and its microbial mutualists. – Molecular Ecology 17: 4480-4488.
- MOREAU, C.S. 2008: Unraveling the evolutionary history of the hyperdiverse ant genus *Pheidole* (Hymenoptera: Formicidae). – Molecular Phylogenetics and Evolution 48: 224-239.
- MOREAU, C.S., BELL, C.D., VILA, R., ARCHIBALD, S.B. & PIERCE, N.E. 2006: Phylogeny of the ants: diversification in the age of angiosperms. – Science 312: 101-104.
- MORITZ, C. & CICERO, C. 2004: DNA barcoding: promises and pitfalls. – Public Library of Science Biology 2: e354.
- OHNISHI, H., IMAI, H.T. & YAMAMOTO, M.T. 2003: Molecular phylogenetic analysis of ant subfamily relationship inferred from rDNA sequences. – Genes & Genetic Systems 78: 419-425.
- OLIVER, T.H., LEATHER, S.R. & COOK, J.M. 2008: Macroevolutionary patterns in the origin of mutualisms involving ants. – Journal of Evolutionary Biology 21: 1597-1608.
- OUELLETTE, G.D., FISHER, B.L. & GIRMAN, D.J. 2006: Molecular systematics of basal subfamilies of ants using 28S rRNA (Hymenoptera: Formicidae). – Molecular Phylogenetics and Evolution 40: 359-369.
- PERRICHOT, V., LACAU, S., NÉRAUDEAU, D. & NEL, A. 2007a: Fossil evidence for the early ant evolution. – Naturwissenschaften 95: 85-90.
- PERRICHOT, V., NÉRAUDEAU, D., NEL, A. & DE PLOËG, G. 2007b: A reassessment of the Cretaceous amber deposits from France and their palaeontological significance. – African Invertebrates 48: 213-227.
- PULQUERIO, M.J.F. & NICHOLS, R.A. 2007: Dates from the molecular clock: how wrong can we be? – Trends in Ecology & Evolution 22: 180-184.
- QUEK, S.P., DAVIES, S.J., ASHTON, P.S., ITINO, T. & PIERCE, N.E. 2007: The geography of diversification in mutualistic ants: a gene's-eye view into the Neogene history of Sundaland rain forests. – Molecular Ecology 16: 2045-2062.
- RABELING, C., BROWN, J.M. & VERHAAGH, M. 2008: Newly discovered sister lineage sheds light on early ant evolution. – Proceedings of the National Academy of Sciences of the United States of America 205: 14913-14917.
- RABELING, C., COVER, S.P., JOHNSON, R.A. & MUELLER, U.G. 2007: A review of the North American species of the fungus-gardening ant genus *Trachymyrmex* (Hymenoptera: Formicidae). – Zootaxa 1664: 1-53.
- RONQUIST, F. 2004: Bayesian inference of character evolution. – Trends in Ecology & Evolution 19: 475-481.
- RUBINOFF, D., CAMERON, S. & WILL, K. 2006: A genomic perspective on the shortcomings of mitochondrial DNA for "barcoding" identification. – Journal of Heredity 97: 581-594.
- RUBINOFF, D. & HOLLAND, B.S. 2005: Between two extremes: mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. – Systematic Biology 54: 952-961.
- RUSSELL, J.A., GOLDMAN-HUERTAS, B.M., MOREAU, C.S., BALDO, L., STAHLHUT, J.K., WERREN, J.H. & PIERCE, N.E. 2009: Host specificity and geographic isolation among *Wolbachia* from ants and butterflies. – Evolution 63: 624-640.
- SAUX, C., FISHER, B.L. & SPICER, G.S. 2004: Dracula ant phylogeny as inferred by nuclear 28S rDNA sequences and implications for ant systematics (Hymenoptera: Formicidae: Amblyoponinae). – Molecular Phylogenetics and Evolution 33: 457-468.
- SCHLICK-STEINER, B.C., STEINER, F.M., SANETRA, M., SEIFERT, B., CHRISTIAN, E. & STAUFFER, C. 2007: Lineage specific evolution of an alternative social strategy in *Tetramorium* ants (Hymenoptera: Formicidae). – Biological Journal of the Linnean Society 91: 247-255.
- SCHLÜNS, E.A., WEGENER, B.J., SCHLÜNS, H., AZUMA, N., ROBSON, S.K.A. & CROZIER, R.H. 2009: Breeding system, colony and population structure in the weaver ant *Oecophylla smaragdina*. – Molecular Ecology 18: 156-167.
- SCHULTZ, T.R. & BRADY, S.G. 2008: Major evolutionary transitions in ant agriculture. – Proceedings of the National Academy of Sciences of the United States of America 105: 5435-5440.
- SEIFERT, B. 2009: Cryptic species in ants (Hymenoptera: Formicidae) revisited: we need a change in the alpha-taxonomic approach. – Myrmecological News 12: 149-166.
- SIRVIO, A., GADAU, J., RUEPELLE, O., LAMATSCH, D., BOOMSMA, J.J., PAMILO, P. & PAGE, R.E. 2006: High recombination frequency creates genotypic diversity in colonies of the leaf-cutting ant *Acromyrmex echinator*. – Journal of Evolutionary Biology 19: 1475-1485.
- SITES, J.W. & MARSHALL, J.C. 2004: Operational criteria for delimiting species. – Annual Review of Ecology, Evolution, and Systematics 35: 199-227.
- SMITH, M.A., FISHER, B.L. & HEBERT, P.D.N. 2005: DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: the ants of Madagascar. – Philosophical Transactions of the Royal Society B - Biological Sciences 360: 1825-1834.
- SOLOMON, S.E., BACCI, M. JR., MARTINS, J. JR., VINHA, G.G. & MUELLER, U.G. 2008: Paleodistributions and comparative molecular phylogeography of leafcutter ants (*Atta* spp.) provide new insight into the origins of Amazonian diversity. – Public Library of Science One 3: e2738.
- SONG, H., BUHAY, J.E., WHITING, M.F. & CRANDALL, K.A. 2008: Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. – Proceedings of the National Academy of Sciences of the United States of America 105: 13486-13491.
- SPAGNA, J.C., VAKIS, A.I., SCHMIDT, C.A., PATEK, S.N., ZHANG, X.D., TSUTSUI, N.D. & SUAREZ, A.V. 2008: Phylogeny, scaling, and the generation of extreme forces in trap-jaw ants. – Journal of Experimental Biology 211: 2358-2368.
- STEINER, F.M., SCHLICK-STEINER, B.C., KONRAD, H., LINKSVAYER, T.A., QUEK, S.P., CHRISTIAN, E., STAUFFER, C. & BUSCHINGER, A. 2006a: Phylogeny and evolutionary history of queen polymorphic *Myrmecina* ants (Hymenoptera: Formicidae). – European Journal of Entomology 103: 619-626.
- STEINER, F.M., SCHLICK-STEINER, B.C., KONRAD, H., MODER, K., CHRISTIAN, E., SEIFERT, B., CROZIER, R.H., STAUFFER, C. & BUSCHINGER, A. 2006b: No sympatric speciation here: multiple data sources show that the ant *Myrmica microrubra* is not a separate species but an alternate reproductive morph of *Myrmica rubra*. – Journal of Evolutionary Biology 19: 777-787.
- TSUTSUI, N.D. & CASE, T.J. 2001: Population genetics and colony structure of the argentine ant (*Linepithema humile*) in its native and introduced ranges. – Evolution 55: 976-985.
- TSUTSUI, N.D., SUAREZ, A.V., SPAGNA, J.C. & JOHNSTON, S. 2008: The evolution of genome size in ants. – BioMed Central Evolutionary Biology 8: e64.
- WARD, P.S. 1994: *Adetomyrma*, an enigmatic new ant genus from Madagascar (Hymenoptera: Formicidae), and its implications for ant phylogeny. – Systematic Entomology 19: 159-175.
- WARD, P.S. & BRADY, S.G. 2003: Phylogeny and biogeography of the ant subfamily Myrmecinae (Hymenoptera: Formicidae). – Invertebrate Systematics 17: 361-386.

- WARD, P.S., BRADY, S.G., FISHER, B.L. & SCHULTZ, T.R. 2005: Assembling the ant "Tree of Life" (Hymenoptera: Formicidae). – *Myrmecologische Nachrichten* 7: 87-90.
- WARD, P.S. & DOWNIE, D.A. 2005: The ant subfamily Pseudomyrmecinae (Hymenoptera: Formicidae): phylogeny and evolution of big-eyed arboreal ants. – *Systematic Entomology* 30: 310-335.
- WETTERER, J.K., SCHULTZ, T.R. & MEIER, R. 1998: Phylogeny of fungus-growing ants (tribe Attini) based on mtDNA sequence and morphology. – *Molecular Phylogenetics and Evolution* 9: 42-47.
- WHEELER, W.M. 1910: *Ants: their structure, development, and behavior*. – Columbia University Press, New York, 663 pp.
- WILD, A.L. 2009: Evolution of the Neotropical ant genus *Linepithema*. – *Systematic Entomology* 34: 49-62.
- WILSON, E.O. 1971: *The insect societies*. – Harvard University Press, Cambridge, MA, 548 pp.
- WILSON, E.O., CARPENTER, F.M. & BROWN, W.L. JR. 1967: The first Mesozoic ants, with the description of a new subfamily. – *Psyche* 74: 1-19.
- WILSON, E.O. & HÖLLDOBLER, B. 2005: The rise of the ants: a phylogenetic and ecological explanation. – *Proceedings of the National Academy of Sciences of the United States of America* 102: 7411-7414.
- WINTER, U. & BUSCHINGER, A. 1986: Genetically mediated queen polymorphism and caste determination in the slave-making ant, *Harpagoxenus sublaevis* (Hymenoptera: Formicidae). – *Entomologia Generalis* 11: 125-137.
- ZUCKERKANDL, E. & PAULING, L.B. 1962: Molecular disease, evolution, and genetic heterogeneity. In: KASHA, M. & PULLMAN, B. (Eds.): *Horizons in biochemistry*. – Academic Press, New York, pp. 189–225.