

# KURZE ORIGINALMITTEILUNGEN

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## Molecular Evidence for a Jurassic Origin of Ants

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The ants, a group of ecologically important insects [1], are commonly found in Oligocene and Miocene ambers, whereas fossils of apparent ant-wasp intermediates are well known from the mid-Cretaceous [2]. Based on this record, it has become commonly accepted that the ants arose no earlier than the late Cretaceous and that the adaptive radiation of extant ant genera took place no later than the start of the Tertiary [2].

The recent discovery of *Cariridris bipetiolata*, a Brazilian fossil from the Aptian of the early Cretaceous which has been placed in the ant subfamily Myrmeciinae [3] (represented today by the *Myrmecia* bulldog ants of Australia and nearby islands, has shown, however, that the ants diversified much earlier than previously thought, with *Cariridris*, an undoubted ant, roughly contemporaneous with the previously known ant-wasp intermediates. In this review, we estimate the time of origin of the ant family using the divergences between mitochondrial DNA sequences from ants of six subfamilies and a vespid wasp. We used mitochondrial cytochrome *b* sequences from *Myrmecia gulosa* and the karyotypically diverse [4] *M. pilosula* group [5] to calibrate the evolutionary rate of this sequence in ants. The evolutionary rate was calibrated using codon positions 1 and 2 only (base composition differences at position 3 indicate a lack of stationarity [6]) under the assumption that nucleotide substitutions follow Kimura's

two-parameter model [7]. A conservative estimate of 124.5 Ma for the age of *Cariridris* was used, this being the lower boundary of the Aptian, i.e., 112–124.5 Ma ago [8]. To check the suitability of the data for estimating divergence times, we applied the relative rate test of Wu and Li [9] (as implemented by Muse and Weir [10]), obtaining the results in Table 1, finding that the data do not violate the assumptions of a molecular clock. Using (1) this age, (2) the evolutionary distances between *Myrmecia* species, and (3) the hierarchical phylogenetic approach, i.e., a method for increasing the precision in estimating the distance between a group of taxa (the *M. pilosula* group) and their out-group (*M. gulosa*) by taking into account both phylogenetic structure and variation in component distances [11], yields an estimate of the evolutionary rate for ants of  $0.165 \pm 0.023\%$  (95% confidence intervals) substitutions per million years.

To estimate the date of origin of the ants, we obtained mitochondrial DNA sequences homologous to those mentioned above (positions 11427–12134 in the honeybee mitochondrial genome [12]) from five further ant subfamilies believed to represent the most divergent lineages, and took the largest divergence value as giving the best estimated of the age of the group. This approach yields an age of  $185 \pm 36$  Ma ago (95% confidence limits derived from the SE of the divergence estimate; Fig. 1). Similar re-

sults follow from an analysis using nonsynonymous substitutions [13]. The larger distances to the wasp, with a relatively narrow spread compared to those between ants, indicate that saturation has not been a serious problem in this calculation. Our finding thus places the origin of the ants in the early Jurassic, at least 70 Ma earlier than recorded by fossils [3], and placing the group within the

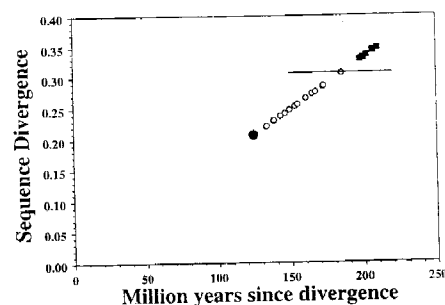


Fig. 1. Sequence divergences and inferred divergence times between *Myrmecia* groups (solid circle), among the ant subfamilies (open circles), and between the wasp *Vespula vulgaris* and the ant subfamilies (solid squares). Divergence times were based on an evolutionary rate which was determined using the evolutionary distances in cytochrome *b* sequence codon positions 1 and 2 between species groups of living *Myrmecia* bulldog ants, and the age of the fossil bulldog ant *Cariridris*. The most ancient divergence between ant subfamilies (here between *Leptomymex* and *Tetraponera*) is taken as the age of the group, with 95% confidence intervals shown (bar). The greater spread of distances between ant subfamilies compared to that between them and the wasp reflects variation in divergence times between ant subfamilies. The *Myrmecia* [5] and *Tetraponera* [18] sequences have been published previously; new sequences, with collection codes and Genbank accession numbers, are from *Camponotus* sp., *nigroaeneus* gp. (ABZI.13, U75351), *Leptomymex unicolor* (ACBW.01, U75354), *Monomorium* sp., *rothsteini* gp. (ABZI.07, U75352), *Rhytidoponera victoriana* (ACAS.01, U75350), and *Vespula vulgaris* (ACAK.01, U75353). Vouchers are placed in the Australian National Insect Collection, Canberra

Table 1. Application of a relative rate test [10] to the sequence data discussed in Fig. 1

	$K_{12}-K_{13}$	SD	Z-statistic
<i>Camponotus-Leptomyrmex</i>	0.019679	0.028972	0.679252
<i>Camponotus-Monomorium</i>	0.003521	0.029811	0.118116
<i>Camponotus-Rhytidoponera</i>	0.015599	0.029412	0.530362
<i>Camponotus-Tetraponera</i>	0.010332	0.031200	0.331156
<i>Camponotus-Myrmecia</i>	0.020518	0.031592	0.649483
<i>Leptomyrmex-Monomorium</i>	-0.016183	0.030166	0.536451
<i>Leptomyrmex-Rhytidoponera</i>	-0.004080	0.029771	0.137061
<i>Leptomyrmex-Tetraponera</i>	-0.009372	0.033361	0.280917
<i>Leptomyrmex-Myrmecia</i>	0.000535	0.031370	0.017050
<i>Monomorium-Rhytidoponera</i>	0.012039	0.029053	0.414388
<i>Monomorium-Tetraponera</i>	0.006811	0.031597	0.215555
<i>Monomorium-Myrmecia</i>	0.017055	0.031735	0.537435
<i>Rhytidoponera-Tetraponera</i>	-0.005228	0.030681	0.170410
<i>Rhytidoponera-Myrmecia</i>	0.007827	0.030746	0.254581
<i>Tetraponera-Myrmecia</i>	0.003423	0.031660	0.108114

The *Vespula* sequence was used as the outgroup. The columns show the difference between the distances to the outgroup, the standard deviation of the difference between the two distances, and the Z-statistic. None of the values approach the significance level ( $Z=1.96$ ) and hence the Bonferroni correction [17] was not necessary. A larger test including an additional 14 *Myrmecia* sequences [5] also showed no detectable departure from a molecular clock.

era of the first hymenopteran fossils [14]. Such a placement suggests that the ants diversified more slowly than previously thought, and that early diversification of the order Hymenoptera may have been more rapid than previously believed. The ants thus arose very early during the breakup of Pangaea [15, 16], indicating that major features of current distributions may reflect the order of separation of the continents.

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## Heterogeneous Turnover of Molecular Organic Substances from Crop Soils as Revealed by $^{13}\text{C}$ Labeling at Natural Abundance with *Zea mays*

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Because of the recent suggestion that the terrestrial biosphere may contain a

missing sink of atmospheric  $\text{CO}_2$  [1], studies on the dynamics of soil organic carbon are of particular interest to try to understand changes in the global carbon cycle [2]. Most investigations build models that consider soil

carbon as the sum of bulk pools of various turnovers [2]. However, a simple consideration of the huge reactivity differences that exist between individual organic substances, such as lipids, amino acids, and carbohydrates, strongly suggests that these compounds may also have significantly different dynamics in soils. If this is the case, models should take into account such heterogeneities at the molecular level. However, the dearth of knowledge on the dynamics of molecular species stems in large part from the extreme complexity of the soil medium and from the lack of suitable methods that allow long-term kinetics of organic species to be eval-

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