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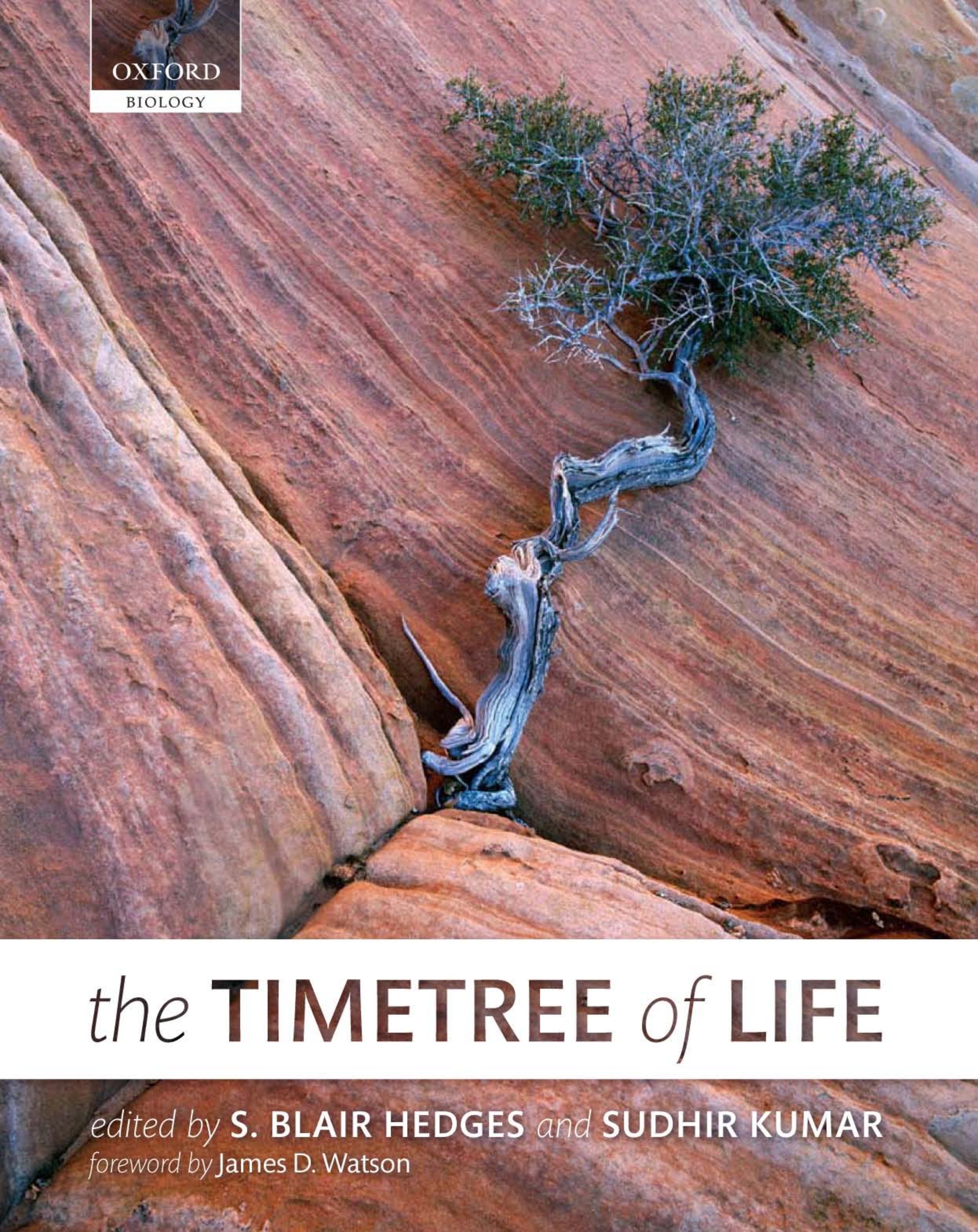

the TIMETREE of LIFE

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Bees, ants, and stinging wasps (Aculeata)

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Abstract

Bees, ants, and stinging wasps comprise the clade Aculeata within the Order Hymenoptera. Molecular dating analyses within Aculeata have focused primarily on ants (Formicidae; ~12,000 species) and bees (Anthophila; ~20,000 species). Published molecular divergence times for ants differ considerably. The most recent study argues for a range of 135–115 million years ago (Ma), consistent with the known fossil record for aculeates. Dating analyses of bees have focused primarily on families containing eusocial species. These studies have revealed that eusociality first evolved ~75 Ma in corbiculate bees and as recently as 20 Ma in several independent halictid taxa.

Aculeate wasps are characterized by the modification of the ovipositor into a sting. All other Hymenoptera deposit their eggs through their ovipositor, while aculeates instead lay their eggs from the base of their sting, and this structure now serves to inject venom into prey and enemies. All members of Aculeata form a monophyletic lineage comprising three superfamilies: Chrysidoidea (seven families), Apoidea (11 families; includes bees and digger wasps; Fig. 1), and Vespoidea (10 families; includes spider wasps, hornets, and ants). Aculeata contains most major groups of eusocial insects, including social wasps, bees, and ants. We review the relationships and divergence times of Aculeata with particular reference to bees and ants, the two taxa that have been the focus of previously published molecular dating studies in this group.

The oldest aculeate fossils are from late Jurassic (146 Ma) compression fossils from Central Asia placed in the Bethylonymidae (*1*). This extinct family probably represents the closest relative of all modern aculeates (*2*). All three modern aculeate superfamilies have an extensive fossil record extending back to the early Cretaceous (140 Ma) (*2*). Early Cretaceous vespoid fossils exist for several families including Sierolomorphidae, Rhopalosomatidae, Vespidae, Scoliidae, and Tiphiidae, but are notably absent for Formicidae (ants). Extinct stem-group lineages to modern ants include Sphecomyrminae (~100–70 Ma) and potentially the more distantly related Armaniidae (~100–75 Ma). The fossil record of Apoidea likewise extends to the early Cretaceous (~140 Ma). Extinct stem-group lineages referred to collectively as Angarosphecidae (*3, 4*) are known from Barremian (140 Ma) up until the early Eocene (54–52 Ma) (*5*) from sites in Europe, South America, and Canada. Bennett and Engel (*6*) provide a recent synopsis of the non-bee apoid fossil record.

There are no published estimates of molecular divergence times among aculeate families. In order to provide a rough molecular timescale for this group, we present analyses of *18S* and *28S* data obtained from GenBank that include representatives of all aculeate families for which these data are currently available (Table 1; Fig. 2).

Fig. 1 A halictid bee (*Agapostemon virescens*) from New York, USA. Photo credit: A. Wild.

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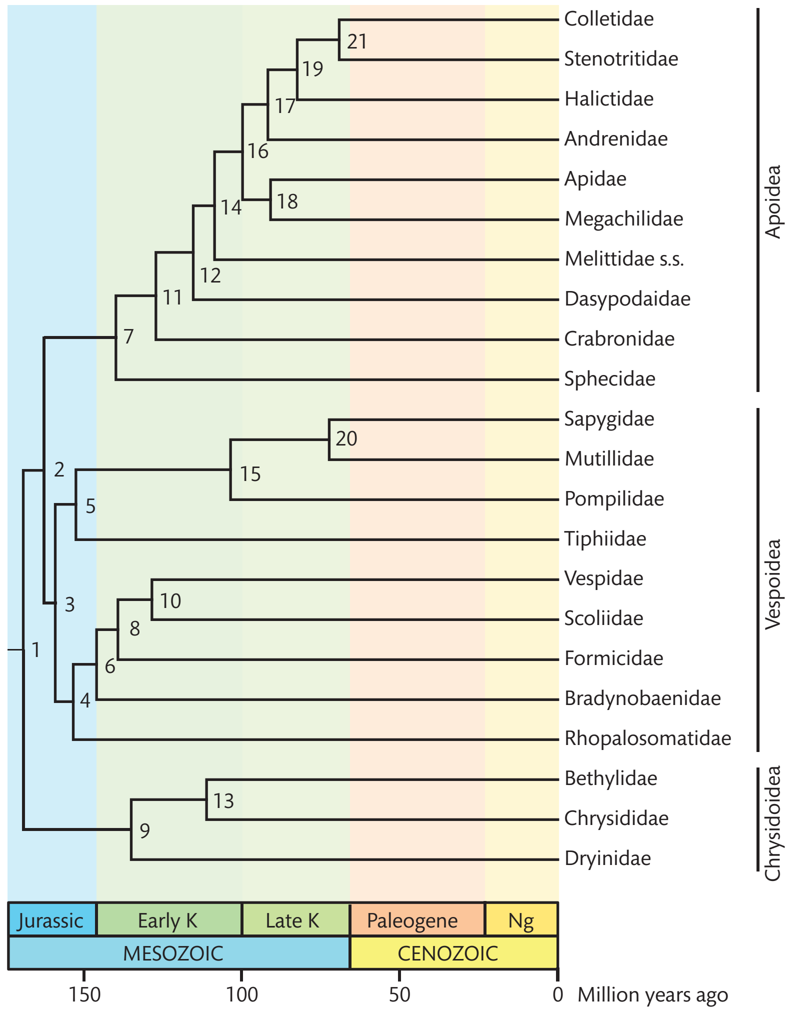

Fig. 2 A timetree of the bees, ants, and stinging wasps (Aculeata). Divergence times are shown in Table 1. *Abbreviations*: Ng (Neogene) and K (Cretaceous).

Our results suggest that the three aculeate superfamilies began to diversify in the middle Jurassic (~170 Ma). Many of the modern vespoid families diverged in the late Jurassic through the early Cretaceous, although a few families including Pompilidae, Mutillidae, and Sapygidae may have originated considerably more recently. Within Apoidea, the clade containing extant bee families arose at least 120–112 Ma, and by the end of the Cretaceous all modern bee families had evolved.

Detailed phylogenetic and molecular divergence dating studies have been published on two groups of aculeates—ants and bees—and we discuss each of these taxa in turn. Ants form the monophyletic Family Formicidae within Vespoidea. All ants are eusocial and collectively occupy keystone positions in many environments (*7, 8*). Most work on higher-level molecular systematics in ants has focused on one or several closely related subfamilies (*9–16*). In 2006, however, two larger phylogenies were published encompassing a much greater portion of ant diversity: Moreau *et al.* (*17*), which included ~4.5 kilobases (kb) from six genes and 19 subfamilies; and Brady *et al.* (*18*), which included ~6 kb from seven genes and 20 subfamilies. All available molecular evidence agrees on a robust group termed the formicoid clade (*19*) that unites 14 of the 20 subfamilies of ants, including several subfamilies containing species displaying presumptive primitive behaviors and morphologies. Bayesian analyses of these large data sets also suggest that Leptanillinae, a rarely encountered and poorly known ant subfamily (*20*), is the closest relative of all other ants in the arrangement

Table 1. Divergence times (Ma) and their confidence/credibility intervals (CI) among bees, ants, and stinging wasps (Aculeata).

Timetree		Estimates			
Node	Time	This study (a)		This study (b)	
		Time	CI	Time	CI
1	170.0	166.1	181–152	173.9	190–159
2	163.5	162.1	176–149	164.9	178–152
3	159.9	158.6	173–146	161.2	174–149
4	154.1	152.1	168–136	156.1	171–142
5	153.2	153.7	168–140	152.8	165–141
6	146.7	142.6	159–126	150.8	167–136
7	140.6	139.5	158–121	141.7	159–125
8	139.9	136.5	153–121	143.2	159–128
9	135.7	136.7	176–84	134.8	180–74
10	129.2	120.5	147–88	137.9	157–117
11	127.8	130.0	148–112	125.6	142–111
12	116.0	120.3	138–103	111.7	126–98
13	111.7	111.7	162-59	–	–
14	109.3	112.1	131–94	106.5	121–92
15	104.2	91.8	138–44	116.6	155–69
16	100.4	103.0	121–85	97.8	113–83
17	92.3	96.4	116–77	88.2	103–74
18	91.4	94.2	114–76	88.5	107–69
19	83.0	88.4	109–68	77.7	92–63
20	72.7	50.1	97–12	95.2	143-45
21	69.6	75.3	102–49	64.0	82-46

Note: Node times in the timetree represent the mean of time estimates from different analyses. Dates were estimated with two independent data sets, *18S rRNA* (a) and *28S rRNA* (b), obtained from the public databases (GenBank) and aligned by hand. The 18S data set included 177 taxa and 770 aligned base pairs (bp) and spanned Symphyta, Apocrita, and Aculeata in the Hymenoptera. The *28S* data set included 157 taxa and 1314 aligned base pair, and spanned Apocrita and Aculeata with a single symphytan species. The *18S* data set was date-calibrated with 19 priors and *28S* with 14 priors, and the data sets were analyzed using an uncorrelated lognormal relaxed clock and a GTR+G+I model of evolution in BEAST 1.4.6 (*59*). Phylogenetic relationships were constrained using information from the most recently published studies available (*32, 60, 61*), with the important caveat that some vespoid relationships are not robust (*62*) and may change with new data.

(Leptanillinae + (formicoids + poneroids)), but other methods of analysis as well as removal of long-branched outgroups demonstrate that alternative relationships at the base of the ant tree are also supported by the current data (*18*). Sampling of additional phylogenetic characters and taxa, as well as more sophisticated methods of dealing with potential phylogenetic artifacts, will be necessary to resolve the earliest relationships among ants with any degree of certainty.

The first ants belonging to modern subfamilies appear in the fossil record ~100 Ma. Although Cretaceous ant fossils are relatively scarce, representatives of several modern subfamilies including Formicinae, Dolichoderinae, and Myrmicinae (all within the formicoid clade) are identifiable from this time period (*2, 21, 22*). By the Eocene ants appeared to have diversified substantially, as indicated by the many modern genera found in Baltic amber (*23, 24*) and other deposits (*25, 26*).

Crozier *et al.* (*27*) represents the first attempt to infer the age of ants using molecular data. Because this study preceded the development of phylogenetic relaxed clock methods, the authors instead used linear regression of mitochondrial DNA pairwise distances, calibrated using the fossil *Cariridris* [which has since been transferred from Formicidae to Ampulicidae (*1*)]. Their analysis suggested a Jurassic origin for ants dated at 185 ± 36 Ma. Although this estimate may be too old, this paper was notable in challenging the notion that the clade of modern ants appeared concomitantly with their first appearance in the fossil record.

Several subsequent dating studies also hinted that modern ants were somewhat older than indicated by their fossil record. Bayesian divergence dating (*28*) studies of army ants and relatives (dorylomorphs) (*10*) and bulldog ants (Myrmeciinae) (*12*) in which the ant node was given an *a priori* value consistently returned older *a posteriori* dates for that node. However, because these studies were designed to date nodes within specific groups of ants and possessed very limited taxon sampling outside these groups, the interpretation of this analytical behavior on the origin of ants is not clear.

The two large-scale molecular ant phylogenies discussed previously (*17, 18*) have also provided the most comprehensive dating estimates currently available for ants. Although both studies used a similar, overlapping set of over 40 ant fossils as minimum-age calibrations and both relied primarily on the same analytical method of penalized likelihood (*29*), these two studies resulted in substantially different age estimates. Moreau *et al.* (*17*) inferred a range of dates for the antiquity of modern ants at 168–140 Ma, with this variation reported as being caused solely by minor (~5 Ma) alterations in the minimum-age calibrations from three recent fossil strata. Brady *et al.* (*18*) inferred a younger range of ~135–115 Ma for the origin of modern ants, and argued that these dates were more accurate for several reasons. Their analyses were not influenced by these minor differences in minimum calibration ages, which altered their estimates by only 0–2 million years. Instead, the range established by Brady *et al.* (*18*) was based on calibrating their basal outgroup node (all sampled Aculeata except Chrysidoidea) with lower and upper bounds using additional information from the entire aculeate fossil record. The range of ~135–115 Ma also accords with arguments based on the overall completeness of the fossil record that modern ants originated ~120 Ma (*2*). These estimates correspond roughly with our own estimates based on a far smaller sample of ant taxa and just two genes. Our data puts the common ancestor of the modern ants plus their older, now extinct relatives (including Sphecomyrminae) at 137–143 Ma (Table 1).

Bees comprise a monophyletic group of ~20,000 species of Aculeata specialized on floral resources such as pollen, nectar, and floral oils. Bees are currently the most important pollinators of angiosperm plants and may have played an important role in angiosperm diversification in the early to mid-Cretaceous. Based on the most recent study (*30*), bees form the closest relative of the spheciform wasp Family Crabronidae. Monophyly of bees is supported by 14 morphological, developmental, and behavioral characters (*31*) and molecular evidence (*32*). Bees are currently divided into seven families: Andrenidae, Colletidae, Halictidae, Melittidae and Stenotritidae (which together comprise the "short-tongued" bees), and Megachilidae and Apidae (which comprise the "long-tongued" bees). Family-level bee phylogeny has been analyzed based on morphology (*33, 34*) as well as combined morphological and molecular data (*32, 35*). While Colletidae has traditionally been viewed as the basal branch of bees, a new view is emerging in which the root node of bees falls near or within the Family Melittidae (*32, 35–37*).

The antiquity of bees remains unclear. There are no published relaxed clock-dating analyses at the level of the bee families, but the fossil record of bees would appear to greatly underestimate their true antiquity. The oldest fossil of a member of the clade of living bees (*Cretotrigona prisca* from New Jersey amber) is closely related to extant stingless bee groups and, in fact, was placed in an extant genus (*Trigona*) when first described (*38, 39*). The antiquity of *C. prisca* is somewhat controversial (*40*). While initially presumed to be 80 Ma (*38, 39*), it has since been estimated to be 70 Ma (*41*) and 65 Ma (*42*) in age. Other important bee fossil deposits include the French (Oise) Eocene amber (~53 Ma), the Baltic amber (~42 Ma), and the Dominican amber (~23 Ma). The Baltic amber includes representatives of the extant families Apidae, Megachilidae, Melittidae, and Halictidae, and one extinct family closely related to Melittidae (Paleomelittidae) (*43*). Dominican amber deposits include representatives of five of the seven extant bee families (Stenotritidae and Melittidae are not represented). The French (Oise) amber includes the oldest fossil melittid (*Paleomacropis eocinicus*) (*37*). This fossil shows oil-collecting hairs typical of extant members of the genus *Macropis*, indicating that oil collecting is an ancient trait in bees (*37*).

Bees were certainly present as far back as 100 Ma because there are well-preserved pemphredonine (crabronid) wasp fossils from the Burmese amber (*44*). The presence of the presumed closest relative of bees in the Burmese amber implies that stem-group bees were also present at that time. A recent report of an apparently pollen-collecting apoid from 100 Ma old Burmese amber (*45*) is consistent with this hypothesis. One could furthermore conjecture that bees could not have arisen before the origin of the angiosperms (~140 Ma) which they pollinate. Therefore, a conservative window for bee origins would be between 100 and 140 Ma, a range of dates consistent with our molecular dating results (Table 1; node 12: 120–112 Ma).

Several studies have used relaxed clock-dating methods to estimate the antiquity of particular bee groups. Such studies have sometimes been hindered by the limited numbers of fossil calibration points available and thus there are large standard errors on some date estimates. Fossil-calibrated phylogenies exist for Xylocopini (*46*), Allodapini (*47*), Bombini (*48*), and Halictidae (*49, 50*).

There is enormous variation in the antiquity of eusocial lineages of bees. Within Halictidae, eusociality appears to have arisen within a narrow, and relatively recent, time period 22–20 Ma (*50*). Several dates exist for the eusocial allodapine bees, a monophyletic group of Old World social and socially parasitic species (*47*), ranging from a mean of 59–35 Ma. Corbiculate bees arose far earlier. The oldest fossil corbiculate is *C. prisca* (65 Ma) but our unpublished molecular estimates place the group close to 75 Ma in age. The origins of eusociality in bees therefore spans a time period from the late Cretaceous to the Miocene.

The origin of ants occurred substantially before any of these bee groups, and their antiquity is matched among social insect taxa only by the termites (Termitidae) (*51*) which originated >130 Ma based on fossil evidence (*52, 53*). There are no published estimates of the antiquity of eusocial Vespidae, another major group of aculeate social insects. A recent molecular phylogeny shows that eusociality evolved twice in this family (*54*), and the vespine fossil nest *Celliforma favosites* indicates that eusociality evolved in one of these groups >63 Ma (*55*).

There appears to be a fairly clear correlation between antiquity and social complexity in the groups of social aculeates for which speciation times have been estimated. Halictid bees, the youngest taxa among all eusocial aculeates, show the smallest colonies, a high frequency of reversion to solitary nesting, and only limited queen/worker dimorphism (*56*). The oldest taxa, corbiculate bees and ants, possess the most elaborate societies with substantial queen/worker dimorphism, advanced forms of communication, colony defense and nest founding, and no known cases of secondarily solitary species (species that have reverted back to solitary nesting from a eusocial ancestor) (*57*). As suggested by Wilson and Hölldobler (*57, 58*), the advanced eusocial groups (such as ants and corbiculate bees) appear to have reached a "point of no return" where eusociality cannot revert to solitary nesting.

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