

# Family-Level Divergences in the Stinging Wasps (Hymenoptera: Aculeata), with Correlations to Angiosperm Diversification

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**Abstract** Diversification in insects has often been linked to the evolution of angiosperms. The majority of studies reporting this link, however, have been done on herbivorous insects. It remains unclear if the diversification of angiosperms was also influential in the diversification of species-rich, carnivorous insect groups. Here we investigate the timing of the origin and diversification in the stinging wasps (Hymenoptera: Aculeata). We employ a Bayesian Markov chain Monte Carlo relaxed clock approach to estimate divergence times for 13 wasp families and eight superfamilies. Divergence times are calibrated with 12 fossils representing groups in various lineages. Our results indicate that many of the modern aculeate families originated during the Cretaceous and in concert with the diversification of angiosperms. This similarity between diversification ages in wasps and in angiosperms may be due to an increased habitat complexity and prey diversity that early angiosperm forests provided.

**Keywords** Vespoidea · Insect diversification · Relaxed molecular clock · Angiosperms

## Introduction

Of the approximately two million eukaryotic species described to date, over half are insects (Footitt and Adler 2009). This diversity, however, is not equally distributed across the class, with nearly 80 % of species being found in only four orders: Coleoptera, Lepidoptera, Diptera, and Hymenoptera (Grimaldi and Engel 2005). High diversity in herbivorous groups is often attributed to co-diversification with angiosperms beginning in the Cretaceous (reviewed by Grimaldi and Engel 2005). Yet, it remains unclear whether the Cretaceous diversification of angiosperms also influenced the radiation of species-rich carnivorous insect groups such as those found in Hymenoptera.

Recently, some authors have suggested that the radiation of angiosperms increased habitat complexity, enabling omnivorous and perhaps carnivorous insect groups to diversify (e.g., ants: Moreau et al. 2006; ground beetles: Ober and Heider 2010). Also, the diversification of herbivorous insects in the Cretaceous may have increased prey availability for carnivorous insects, enabling diversification (Moreau et al. 2006). While these studies have provided insights into Cretaceous diversification in insects, more work is needed in order to understand the timing of diversification in carnivorous insects.

One of the most diverse and easily recognizable groups of carnivorous insects is the stinging wasps (Hymenoptera: Aculeata). Aculeata comprises eight superfamilies (Pilgrim et al. 2008) with an estimated diversity of over 77,000 species worldwide (Goulet and Huber 1993). While some aculeate groups have shifted from an ancestral carnivorous diet to using plant resources, the majority of aculeate species are parasitoids or predators of various arthropods. Because of their unique ecology, bees and ants have received the most attention from evolutionary biologists,

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and diversification in both groups has been linked to the late Cretaceous radiation of angiosperms (Grimaldi 1999; Danforth et al. 2004; Moreau et al. 2006; but see Pie and Tschá 2009). Divergence times for the remaining aculeate families remain poorly known. Grimaldi and Engel (2005) reviewed the fossil record of Aculeata, suggesting that many aculeate superfamilies may have originated near the Jurassic/Cretaceous (J/K) boundary, but this remains untested using molecular phylogenetic data.

Comparisons of diversification times among versus within aculeate families and superfamilies could provide further insights into the influence of angiosperm radiations. Diversification may have first occurred in low trophic levels (i.e., angiosperms) followed by radiations in subsequent trophic levels (i.e., herbivores and carnivores). This possibility has been referred to as a phylogenetic cascade (Forister and Feldman 2011). Diversification in different trophic levels may have occurred in concert, with radiations in all trophic levels co-occurring, or there could have been prolonged periods of evolutionary stasis between subsequent diversification (phylogenetic fuse: Cooper and Fortey 1998). Conversely, rather than a phylogenetic cascade beginning with the diversification of angiosperms lighting the fuse leading to explosive radiations in insects, carnivorous wasps might have diversified before angiosperms in response to factors other than ecological interactions, such as repeated geographic isolation.

To determine whether the stinging wasps diversified before angiosperms, concurrently with angiosperms, or temporally lagged behind angiosperm radiations, we conducted molecular dating analyses based on the family-level designations proposed by Pilgrim et al. (2008) calibrated with 12 fossils of various ages.

## Materials and Methods

Pilgrim et al. (2008) estimated phylogenetic patterns within Aculeata through analyses of a four-gene dataset (EF1- $\alpha$ , 28S, long-wavelength rhodopsin, and wingless) consisting of 2693 total base pairs. Our aim in this paper is not to evaluate the phylogenetic results of Pilgrim et al. (2008), nor is it to reconstruct the phylogenetic patterns among aculeate families, but rather, our goal is to estimate divergence dates and rates of diversification for the groups presented by these authors.

The aligned sequence data from Pilgrim et al. (2008) was used to estimate divergence dates under a Bayesian relaxed clock approach to divergence time estimation using BEAST 1.6.2 (Drummond and Rambaut 2007). The program BEAUti 1.6.2 (Drummond and Rambaut 2007) was used to generate the file used in BEAST. A Yule process speciation prior was implemented and the GTR + I +  $\Gamma$

was applied with each gene partitioned separately, with base frequencies estimated during the analysis. An uncorrelated lognormal model was applied to estimate the relaxed molecular clock. The analysis was run for 50,000,000 generations and sampled every 1,000 generations with the tree generated by Pilgrim et al. (2008) used as a starting tree. A burn-in of 10 % was removed after convergence and diagnostic plots were assessed using Tracer 1.5 (Rambaut and Drummond 2007). The 50 % consensus of post-burnin trees and nodal posterior probabilities (PP) were calculated with TreeAnnotator 1.6.2 (Rambaut and Drummond 2007). In addition, the analysis was also run using the SRD06 model (Shapiro et al. 2006) with all parameters unlinked across partitions and loci. To account for potential topological differences in the tree resulting from our BEAST analysis and the phylogeny presented by Pilgrim et al. (2008), we ran an additional analysis with all family-level relationships constrained to mirror those of Pilgrim et al. (2008).

Each BEAST analysis was calibrated with 12 fossils from various aculeate families and different ages (Table 1). An attempt was made to use the oldest fossil known for each group, according to Grimaldi and Engel (2005). To constrain nodes with minimum ages, all fossil calibration points were designated as stem-group ages and were placed at the base of the stem leading to the taxa they represent (i.e., the node where the common ancestor of the group split from the ancestor of the sister group) and were assigned a log-normal prior with a zero offset based on the fossil age (Table 1) and a SD of 1.

A second molecular dating analysis was implemented in r8s 1.71 (Sanderson 2002), which uses a penalized likelihood approach to rate smoothing. The consensus tree that resulted from the unconstrained BEAST analyses was used in the r8s analysis with the same calibration points as previous analyses assigned as minimum ages and the root fixed at 147 Ma based on the estimated age of the group from previous analyses. The penalized-likelihood method with the truncated Newton algorithm was implemented to estimate rates and divergence dates. Lineage through time plots were constructed from phylogenetic trees using the *Ape* package in the Program R.

The diet of each family treated in this analysis is based on Goulet and Huber (1993). In the following results and discussion, the ages are based on the date each lineage diverged from its sister lineage (i.e., the age of the stem group).

## Results and Discussion

While the timing of diversification has been examined for some aculeate groups (ants: e.g., Moreau et al. 2006; Brady et al. 2006; bees: e.g., Danforth et al. 2004; Poinar and

**Table 1** Information on the fossils used to calibrate the molecular dating analysis

Node number	Fossil family or subfamily	Fossil species	References	Fossil age (Ma)
1	Bethylidae	<i>Apenesia electriphila</i>	Cockerell (1917)	100
2	Rhopalosomatidae	<i>Mesorhopalosoma cearae</i>	Grimaldi et al. (1990)	92
3	Euparagiinae	<i>Curiosivespa derivata</i>	Carpenter and Rasnitsyn (1990)	100
4	Formicinae	<i>Kyromyrma neffi</i>	Grimaldi and Agosti (2000)	90
5	Apiformes	<i>Melittosphex burmensis</i>	Poinar and Danforth (2006)	100
6	Apidae	<i>Cretotrigona prisca</i>	Michener and Grimaldi (1988), Engel (2000)	70
7	Halictidae	<i>Corimbatichnus fernandesi</i>	Genise and Verde (2000)	60
8	Sierolomorphidae	<i>Loreisomorpha nascimbenei</i>	Rasnitsyn (2000)	100
9	Myrmosidae	<i>Protomutilla</i> sp.	Lelej (1986)	45
10	Ephuta	<i>Ephuta calavigera</i>	Brothers (2003)	25
11	Pompilidae	Pompilidae sp.	Weitschat and Wichard (2002)	45
12	Myzinae	<i>Geotiphia orientalis</i>	Rasnitsyn (1986)	30

Node numbers correspond to those in Fig. 1

Danforth 2006; Danforth and Poinar 2011; Michez et al. 2012), molecular-based divergence dates have been untested for most wasp families (but see Brady et al. 2009). Our analyses provide these estimates for 13 aculeate wasp families, as well as the superfamilies Chrysoidea and Apoidea (Table 2; Fig. 1). The phylogenetic reconstruction from the unconstrained BEAST analysis resulted in a well-supported tree (Fig. 1) with a similar topology to the tree presented by Pilgrim et al. (2008). The constrained BEAST analysis resulted in similar age estimates for all families except Formicidae, Pompilidae, and Scoliidae, due primarily to topological differences (Table 2). Additionally, the BEAST analysis using the SDR06 model also suggested similar divergence dates (within 5 million years for all nodes). The r8s analysis resulted in similar age estimates as the unconstrained BEAST analysis (Table 2).

Although we were able to include only a fraction of the species diversity in Aculeata families (e.g., we included six of the ca. 5,000 species of Mutillidae), our age estimates are similar to accepted ages for those groups that have been previously studied. For example, Grimaldi and Engel (2005) estimated the age of the common ancestor of extant aculeate groups to be ~155 Ma, which is similar to our estimated age of 147 Ma. Also, our estimate of 118 Ma for the origin of ants agrees with several recent studies (Grimaldi and Engel 2005; Brady et al. 2006). In addition to the similarity of our date estimates to previous estimates, some recent divergences in our analysis also match previously published dates. The split between *Sphaerophthalma* and *Dilophotopsis* in Mutillidae, for example, is estimated to be 12.9 Ma, which is similar to an estimated age of 16 Ma given by Pitts et al. (2010).

While the age of angiosperms based on fossil data is 125 Ma, molecular data suggest the group may be older (reviewed by Bell et al. 2010). Regardless of the origin of angiosperms, this group is thought to have experienced rapid diversification during the late Cretaceous (e.g., Schneider et al. 2004). Our divergence date estimates for aculeate families indicate that these groups may have originated during the period of angiosperm diversification (Table 2; Fig. 2).

The origins of many aculeate groups are estimated to fall within the generally accepted time frame in which angiosperms were becoming dominant (Table 2; Fig. 2). While our sampling enables us to determine the age of each wasp family, for most families we are lacking the internal sampling density required to determine patterns of diversification at a finer scale.

Our age estimates for aculeate wasps suggest that diversification of angiosperms could have facilitated diversification in carnivorous wasps. Although there is not a dramatic increase in aculeate diversification rates during early angiosperm diversification, the origin of many wasp families is temporally connected to the time period when angiosperms were diversifying indicating some connection between the two groups. However, due to the predatory lifestyles in many of these aculeate families, wasp diversification may have been indirectly influenced by the evolution of angiosperms, as compared to the more direct influence of angiosperms as host plants for species-rich herbivorous insect groups. For many wasp families, the rise of angiosperms may have provided increased habitat complexity that facilitated the exploitation of new nesting habitats, possibly promoting speciation. Furthermore, the

**Table 2** Estimated age in million years (Ma) with 95 % HPD credibility intervals for Aculeata families and superfamilies as designated in Pilgrim et al. (2008)

Family or superfamily	Age (Ma) and 95 % HPD from BEAST	Age (Ma) from r8s	Age (Ma) and 95 % HPD from BEAST*	Diet
Bradynobaenidae	103 (85–120)	99	103 (81–120)	Unknown
Chyphotidae	98 (83–114)	94	93 (77–114)	Unknown
Formicidae (ants)	118 (106–132)	114	134 (122–147)	Omnivorous
Mutillidae (velvet ants)	85 (69–102)	82	85 (65–105)	Primarily Apoidea
Myrmosidae	47 (45–75)	45	48 (45–78)	Apoidea
Pompilidae (spider wasps)	85 (69–102)	82	97 (77–117)	Spiders
Rhopalosomatidae	120 (111–133)	115	123 (112–135)	Crickets
Sapygidae	47 (45–75)	45	48 (45–78)	Apoidea
Scoliidae	103 (85–120)	99	110 (89–127)	Scarab beetles
Sierolomorphidae	101 (100–105)	97	101 (100–106)	Unknown
Thynnidae	98 (83–114)	94	93 (77–110)	Soil dwelling beetles
Tiphiidae	101 (100–105)	97	101 (100–106)	Soil dwelling beetles
Vespidae (paper wasps, hornets, and yellow jackets)	120 (111–133)	115	123 (112–135)	Generalist predators
Apoidea (bees and hunting wasps)	130 (118–142)	125	126 (114–138)	N/A
Chrysoidea	147 (134–164)	147	147 (134–162)	N/A
Formicoidea	118 (106–132)	118	134 (122–147)	N/A
Pompiloidea	116 (102–131)	116	116 (101–132)	N/A
Scolioidea	118 (106–132)	118	125 (114–138)	N/A
Thynnoidea	116 (102–131)	116	116 (101–132)	N/A
Tiphioidea	126 (113–141)	125	124 (110–140)	N/A
Vespoidea	145 (132–161)	145	145 (132–160)	N/A

Ages from three analyses are shown: a topologically unconstrained BEAST analysis, a r8s analysis, and a BEAST analysis constrained to match the topology of Pilgrim et al. (2008). Where known, the common name and diet are given for each family based on the descriptions from Goulet and Huber (1993)

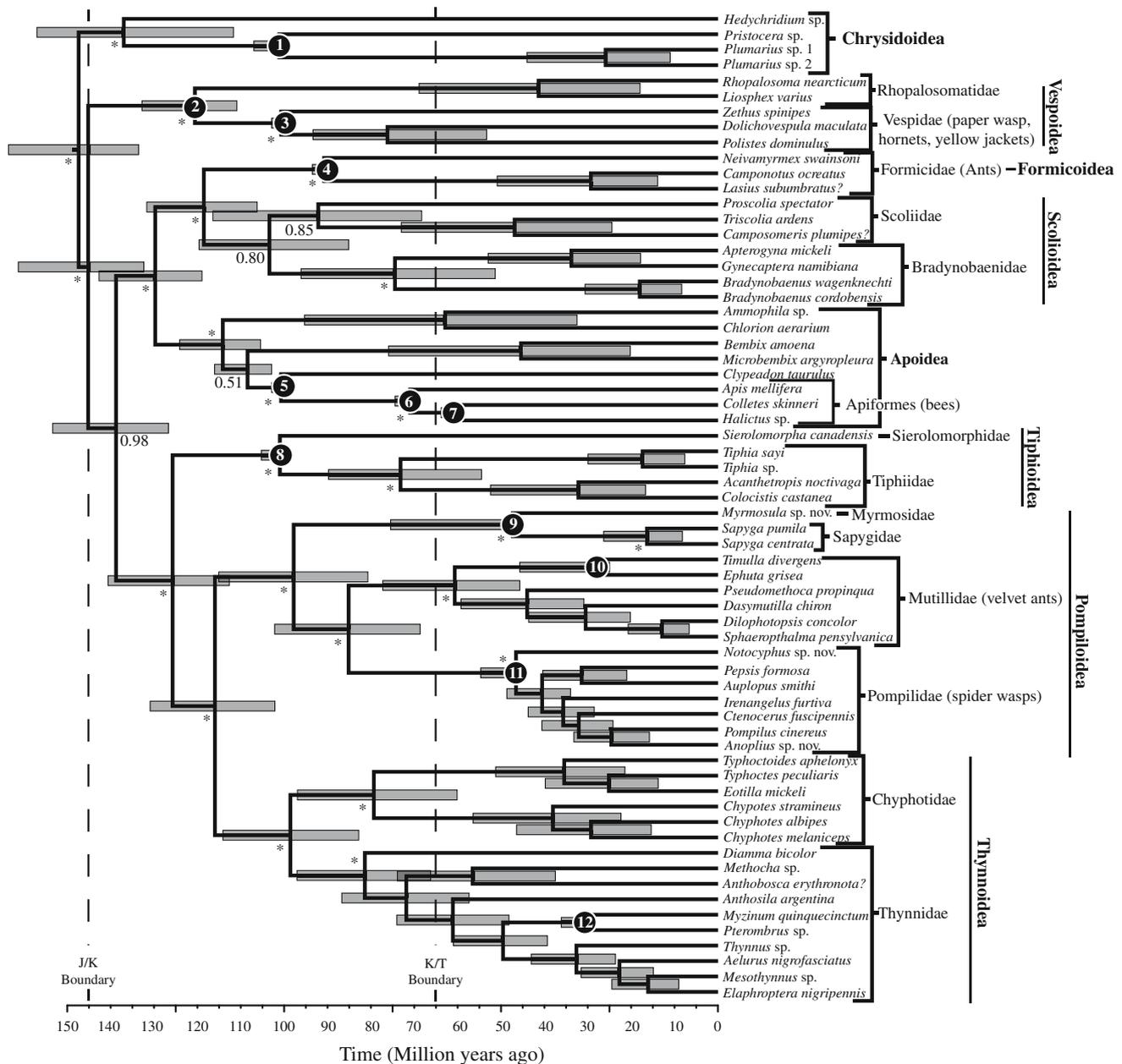
\* Indicates the analysis in which the data were constrained to the Pilgrim et al. (2008) phylogeny

rise of angiosperms and the correlated rise in herbivorous insects undoubtedly provided increased prey diversity and may have been influential in diversification, particularly in those families with generalist predators and those whose members feed primarily on herbivorous insects (see Table 2).

Some noteworthy examples of how increased prey diversity may have influenced predatory wasp diversity can be derived from our analysis. For one example, three of the youngest wasp families in our analysis, Mutillidae, Sapygidae, and Myrmosidae are primarily parasitic on members of the superfamily Apoidea (Table 2). Based on our data, Apoidea originated around 130 Ma (95 % HPD 118–142 Ma) and began diversifying soon after (Fig. 1). The aforementioned wasp families that parasitize Apoidea

did not appear until over 40 million years later. Diversification in Apoidea has been linked to the rise of angiosperms (Grimaldi 1999; Danforth et al. 2004) and, therefore, it seems probable that Mutillidae, Sapygidae, and Myrmosidae diversified in an indirect response to angiosperm radiation, as a response to the increase in angiosperm-feeding prey diversity.

Wasps in the family Pompilidae feed exclusively on spiders. While not directly associated with angiosperms, Pompilidae also may have diversified in response to increased prey diversity. Pompilidae diverged from its sister lineage at 85 Ma (95 % HPD 69–102 Ma), and our analysis (which includes all extant subfamilies) suggests crown-group diversification occurred beginning in the early Paleogene (~47 Ma; Fig. 1). Although spiders are an



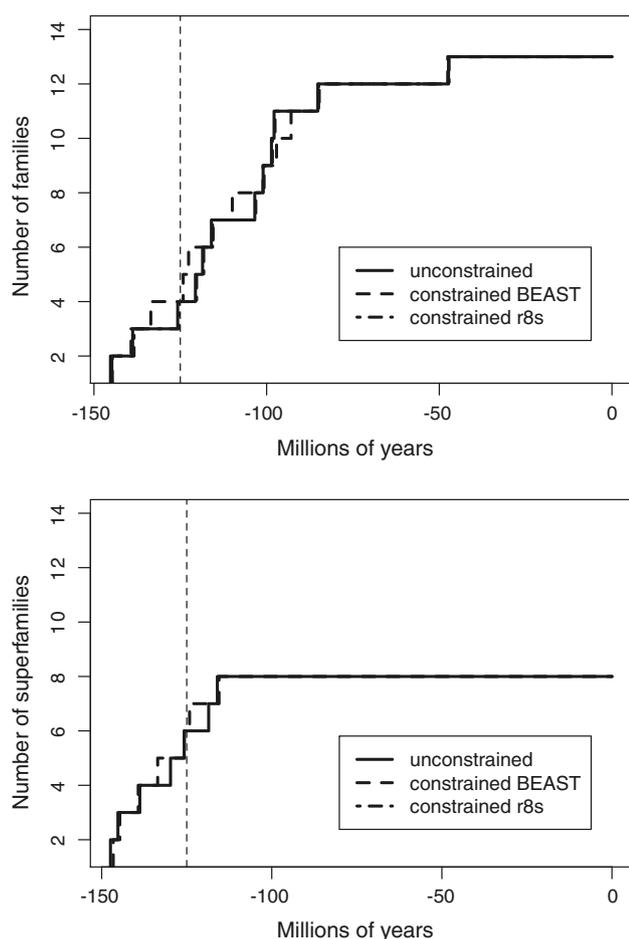
**Fig. 1** Chronogram of aculeate families and superfamilies from a relaxed-clock analysis in BEAST with no topological constraints. Fossil calibration points are marked with *black circles containing numbers*, which correspond to Table 1. Posterior probabilities are shown for families and inter-family relationships, with *asterisk*

representing a posterior probability of 1.0. *Grey bars* at each node represent 95 % highest posterior density (HPD) credibility intervals. The Jurassic/Cretaceous boundary (J/K), and Cretaceous/Paleogene boundary (K/T) are marked

ancient group that originated long before Pompilidae and increased exponentially since their origin in the Mid-Devonian (Selden et al. 1991), analyses of spider fossils show that family-level diversity nearly doubled between 65 and 45 Ma (Penney 2004). This increase in spider diversity could explain the diversification of pompilid wasps.

Several studies have investigated the link between diversification of angiosperms and diversification of

herbivorous insects. This study serves as one of the first to investigate the age of diversification among species-rich groups of predatory insects. Our findings indicate that many wasp families did diversify during the rise of angiosperms. This supports the ideas that increased angiosperm diversity provided increased habitat complexity and increased prey diversity, which may have been the principal factors driving diversification in predatory wasps.



**Fig. 2** Lineage through time plots of families and superfamilies with the oldest angiosperm fossil marked with a dashed vertical line

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