

## Evolutionary Transitions In Aphidiinae (Hymenoptera: Braconidae)

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### Introduction

Aphidiine wasps (Braconidae: Aphidiinae) are solitary koinobiont endoparasitoids of aphids. The subfamily is currently considered to include approximately 50 genera and 400 species which are divided into four tribes: Aclitini, Aphidiini, Ephedrini and Praini (Mackauer & Stary 1967; Stary 1988). The Aphidiini is the largest of the four tribes and includes a majority of known genera and species; it is sub-divided into three subtribes, Aphidiina, Monoctonina and Trioxina. Although aphidiines are defined by a number of synapomorphies (e.g. host specialisation and presence of a flexible suture between the second and third tergites of the gaster), significant differences exist in morphology, biology and behaviour among tribes, genera and species. Because of varied interpretation of these differences, there have been disagreements concerning the phylogenetic placement of some aphidiine taxa.

A number of different phylogenies have been proposed previously for aphidiines based on embryology, morphology and DNA sequences that differ in the postulated relationships among taxa (see Smith *et al.* 1999 for a review of the various phylogenetic proposals for aphidiines]. For example, each of the four tribes mentioned above have been suggested as being basal in independent studies of morphology (Mackauer 1961, 1968; Tobias 1967; Tobias & Kyriak 1971; Edson & Vinson 1979; Chou 1984; Gärdenfors 1986; Finlayson 1990) and DNA (Belshaw & Quicke 1997; Smith *et al.* 1999; Kambhampati *et al.* in press). Determination of which group is basal is important because of its strong implications for ingroup character state polarisation.

Recently, Smith *et al.* (1999) and Kambhampati *et al.* (in press) examined the phylogenetic relationship among aphidiine genera. Smith *et al.* (1999) proposed a phylogenetic tree for Aphidiinae based on 465 bp of the mitochondrial NADH1 dehydrogenase gene. Their study included 39 taxa representing 14 genera and three braconid outgroups. The length of the NADH1 dehydrogenase gene fragment was invariant among the 39 taxa and alignment of the sequence was straight-forward. Smith *et al.* (1999) found that Praini was basal, with strong quantitative support among aphidiines included in their study. However, the study of Smith *et al.* (1999) did not include a potentially basal genus, *Aclitus* Foerster, which possesses a number of presumed plesiomorphic characters (Mackauer 1961; Takada & Shiga 1974).

Kambhampati *et al.* (in press) proposed a phylogenetic tree for aphidiines based on DNA sequence of a portion of the mitochondrial 16S rDNA gene for 47 ingroup taxa representing 24 genera and seven outgroup taxa. Their study indicated the following relationships: *Aclitus* + (Praini + (Ephedrini + Aphidiini)). Although the genus *Aclitus* was basal, it was only represented by a single species (*A. obscuripennis* Foerster) and its position did not have strong quantitative

support. The finding of Kambhampati *et al.* (in press) that Praini was basal relative to Ephedrini is congruent with the results reported by Smith *et al.* (1999). A combined analysis of the NADH1 and 16S rDNA data partitions for 27 ingroup taxa representing 14 genera (*Aclitus* not included) by Kambhampati *et al.* (in press) indicated a topology nearly identical to that which was reported by Smith *et al.* (1999), but with even stronger quantitative support.

In the present study we assess whether evolutionary transitions of various morphological and behavioural character states are compatible with the phylogenetic tree inferred from a combined analysis of the mitochondrial NADH1 and 16S rDNA genes by Kambhampati *et al.* (in press). The mapping of various characters onto the combined evidence tree indicated that many of these characters have a simple evolutionary trajectory, with either a single transition in the case of two-state characters or multiple sequential transitions in the case of multi-state characters.

## Materials And Methods

A list of taxa and the phylogenetic tree on which this study is based was presented by Kambhampati *et al.* (in press). The phylogenetic tree was inferred using maximum parsimony methods in PAUP\* Ver 4d64 (written by D. L. Swofford). Branch support was assessed by bootstrapping (fast stepwise addition-10,000 replications; Felsenstein 1985) and decay index (Bremer 1994). MacClade v. 3.04 (Maddison & Maddison 1992) was used to examine the evolutionary transitions of the following characters by mapping each state onto the phylogenetic tree: A) egg shape, B) shape of first instar mandibles, C) distribution of first instar abdominal spines, D) final instar hypostomal spur, E) venom apparatus, F) shape of ovipositor sheaths, G) pupation behaviour, and H) emergence hole position. These eight characters were selected because they represent a range in morphology from egg to larva to adult, and a range in behaviour from immediately prior to pupation to immediately following pupal development.

## Results And Discussion

### Egg shape and first instar larval structures

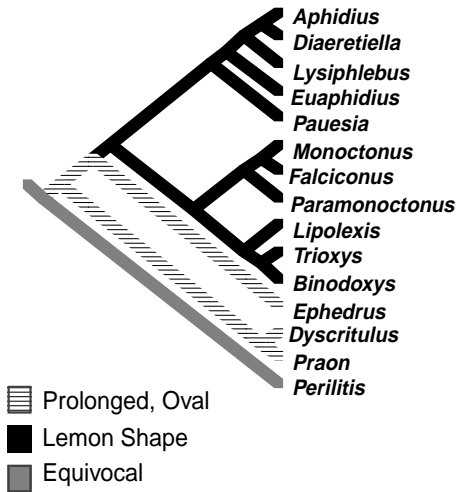
Tremblay and Calvert (1971) examined the systematic position of aphidiines based on embryology. In their study they identified two distinct differences in egg shape associated with aphidiines, those that are prolongately oval and those that are lemon shaped. Mapping each state onto our phylogenetic tree indicated that prolongately oval eggs constitutes the plesiomorphic state, and lemon-shaped eggs, in Aphidiini, the apomorphic state (Fig. 1A).

Similarly, first instar larval aphidiines were examined for differences in morphology by O'Donnell (1989). Two characters associated with the head and abdomen (i.e., mandible shape and abdominal spine distribution) were identified to each have two distinct states. With respect to mandible shape, O'Donnell (1989) found that the first instar larvae exhibited either hook-shaped mandibles or sickle-shaped mandibles; and that the abdominal spines were arranged either in a continuous or discontinuous fashion (i.e. no apparent pattern to arrangement). Our phylogenetic tree indicated two identical evolutionary lineages with respect to the two characters (Figs 1B, 1C). In this regard, hook-shaped mandibles and a continuous arrangement of the abdominal spines are plesiomorphic with a single evolutionary transition for both characters in Aphidiini to sickle-shaped mandibles and a discontinuous arrangement of the abdominal spines.

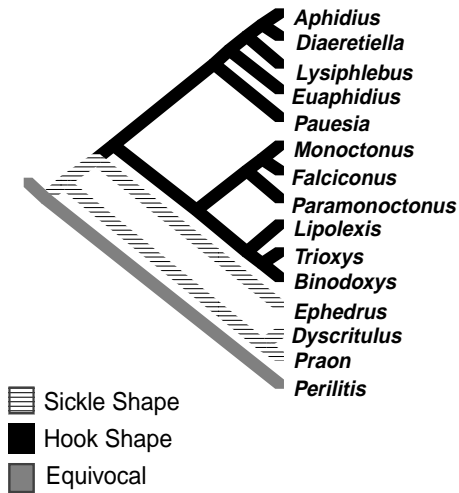
### Fourth instar hypostomal spur

Finlayson (1990) conducted a systematic study of aphidiines in which various cephalic structures of fourth instar larvae were examined for differences. Of particular interest is the presence/

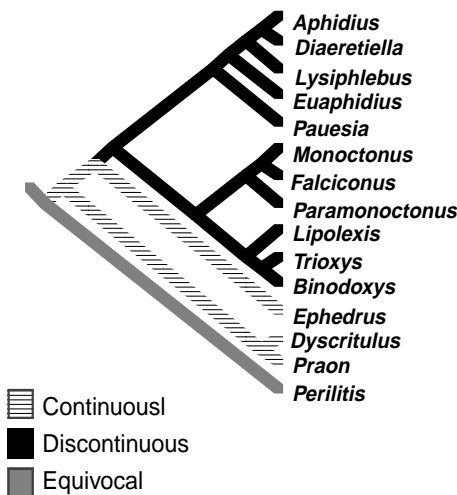
(A) Egg Shape



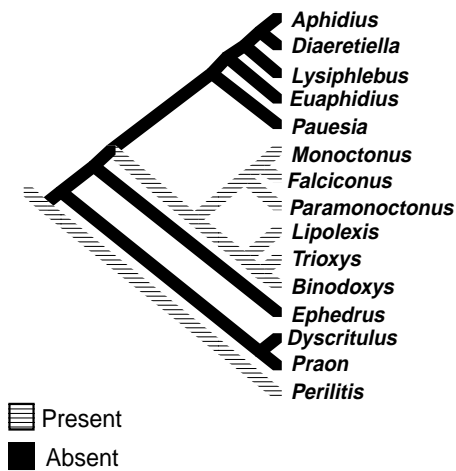
(B) 1st Instar Mandible Shape



(C) 1st Instar Abdominal Spine Distribution



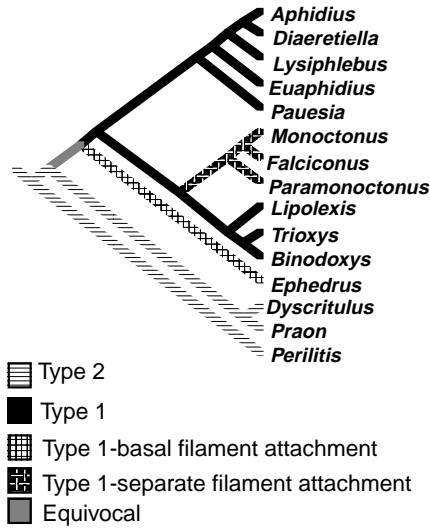
(D) 4th Instar Hypostomal Spur



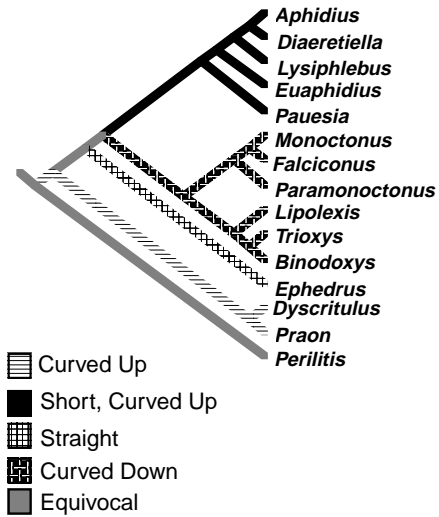
**Figure 1** A phylogenetic tree for Aphidiinae based on DNA sequence of portions of the 16S rDNA and NADH1 dehydrogenase gene. The tree shown is a majority rule consensus of two equally parsimonious trees for 27 aphidiine taxa representing 14 genera and an outgroup (monophyletic genera reduced to a single taxon name). The evolutionary transitions of the following characters were examined: A) egg shape; B) first instar mandible shape; C) first instar abdominal spine distribution; D) fourth instar hypostomal spur;

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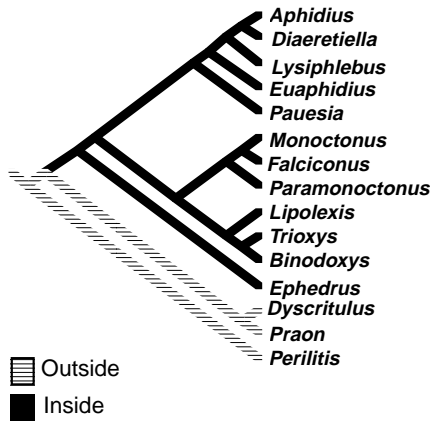
(E) Venom Apparatus



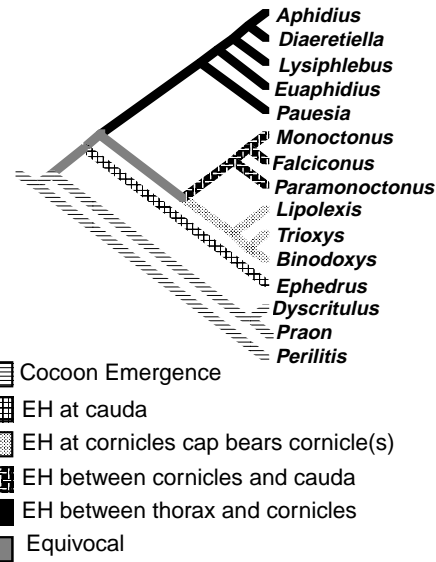
(F) Ovipositor Sheath Shape



(G) Pupation Behavior



(H) Emergence Hole Position



**Figure 1** E) venom apparatus; F) ovipositor sheath shape; G) pupation behavior, and H) emergence hole position. These characters represent a range in morphology from egg to larva to adult and a range in behavior from immediately prior to undergoing pupation to immediately following pupal development.

absence of a hypostomal spur. Finlayson (1990) found that only a sub-set of aphidiine genera (i.e. the Trioxina) possess this structure. Since most braconids possess a hypostomal spur, Finlayson (1990) reasoned that those aphidiines that have lost this structure are more "derived" (i.e. she assumed the sister group to aphidiines possessed a hypostomal spur and that possession of this character is the apomorphic condition). If Finlayson's (1990) hypothesis of aphidiine evolution is correct, it would imply that there has been a considerable amount of convergence during the course of aphidiine evolution (e.g. egg shape, first instar mandible shape, and abdominal spine distribution). In marked contrast, our phylogenetic tree indicates that there was an initial loss and secondary acquisition of this character within a monophyletic group of genera within the Aphidiini (Fig. 1D). Although our explanation is less parsimonious than Finlayson's (1990), it requires only one additional step and is more parsimonious when the evolutionary transitions of many other characters are considered.

### Venom apparatus

Braconid wasps exhibit one of two different types of venom apparatus (VA) morphology following the classification scheme of Edson and Vinson (1979). In general, the VA consists of a venom reservoir, gland filaments, and a venom duct which extends into the ovipositor. Following Edson and Vinson's (1979) classification scheme, one VA type (Type 1) is characterised by a cone shaped reservoir surrounded by many muscles and two or more gland filaments, and is associated with ecto- or endo-parasitoids which pupate inside the host remains. The Type 1 VA can be further sub-divided based on structure (e.g. attachment site of the gland filaments and/or appearance of the venom reservoir). A second type of VA (Type 2) is characterised by a thin walled reservoir surrounded by few muscles and only two gland filaments, and is associated with endoparasitoids which pupate outside the host remains. Unlike other braconid subfamilies which are monomorphic for either type, the Aphidiinae exhibit both types. The VA classification scheme of Edson and Vinson (1979) is highly compatible with our phylogenetic tree with four evolutionary lineages being recognised (Fig. 1E).

The plesiomorphic VA structure within Aphidiinae is a Type 2 structure with no apparent reservoir and is exhibited by members of the tribe Praini which pupate outside and underneath the exoskeleton of the host (Fig. 1E). The apomorphic state is a Type 1 structure and is exhibited by members of Ephedrini and Aphidiini, both of which pupate inside the host. However, the Type 1 VA of Ephedrini differs from that of Aphidiini in that the gland filaments are attached at the base of the venom reservoir and the cuticular lining of venom reservoir lacks a spiral-like appearance. Within Aphidiini, the genera *Monoctonus* Haliday, *Falciconus* Mackauer and *Paramonotonus* Stary (= *Monoctonina*) can be further distinguished by the separate attachment of the two gland filaments to the venom reservoir and the cuticular lining of the venom reservoir lacks a spiral-like appearance (Fig. 1E).

### Ovipositor sheath shape

Female aphidiines exhibit variation in the shape and length of the ovipositor sheaths (3rd valvulae). The ovipositor sheaths can be sparsely or densely pubescent, but they always bear several sensory hairs at their apex. The variation in ovipositor sheath shape ranges from short to long and either straight, or curved up or down. According to Stary (1981), the ovipositor sheaths and their functional differentiation are one of the key characters in the evolution of aphidiine groups. Our phylogenetic tree indicates the presence of four evolutionary lineages with respect to shape and size of the ovipositor sheaths (Fig. 1F). The morphological features of the ovipositor sheaths

are apparent adaptive strategies in parasitoid/host interactions. For example, the downward curved ovipositor sheaths of *Monoctonus*, *Falciconus*, *Paramonctonus*, *Lipolexis* Foerster, *Trioxys* Haliday, and *Binodoxys* Mackauer apparently work in conjunction with a pair of prongs on the last abdominal sternum to hold the attacked aphid and prevent its escape (Stary 1981).

#### Pupation behaviour

Following the completion of larval development, aphidiines will pupate either inside the dead host (most extant species) or outside and underneath the empty exoskeleton of the host (most members of Praini). Mackauer (1961) proposed that outside pupation in Praini evolved by way of secondary loss of internal pupation and may represent an adaptation in response to hyperparasitoids. Our results indicated that this behaviour is plesiomorphic with a single transition to internal pupation among members of Ephedrini and Aphidiini (Fig. 1G). In contrast, Mackauer's (1961) proposal would imply multiple transitions (i.e. from internal pupation in Ephedrini to external pupation in Praini and then back to internal pupation in Aphidiini). It is possible, however, that internal pupation is plesiomorphic despite which aphidiine lineage is basal. Central to this issue is the phylogenetic position of the genus *Areopraon* Mackauer (a rare genus currently classified in Praini not included in our study) which contains species which pupate internally and others which pupate externally or both depending on the circumstances (Stary 1970). If an internally pupating *Areopraon* species is basal to all other Praini, then Mackauer's (1961) proposal of internal pupation being plesiomorphic is possible and would be the most parsimonious explanation as it implies only a single transition. However, the relationship of *Areopraon* to other aphidiines (including Praini) remains to be vigorously tested with cladistic analysis.

#### Emergence hole position

Following the completion of immature development, the adult aphidiine uses its mandibles to cut an emergence hole in the cocoon (Praini) or host exoskeleton (Aclitini, Aphidiini, Ephedrini). Among the aphidiines that pupate inside the host, some species cut an emergence hole between the thorax and cornicles, some species cut an emergence hole at the level of the cornicles (in such case the cap may contain one or both cornicles), some species cut an emergence hole posterior to the cornicles (in such case the cap never contains a cornicle), and finally some species cut an emergence hole perpendicular to the longitudinal axis of the host exoskeleton (in such case the cap bears the cauda and is relatively large; Stary 1970). With respect to emergence hole position, our phylogenetic tree indicates the presence of four distinct evolutionary lineages (Fig. 1H). Of those aphidiines that cut an emergence hole in the exoskeleton of the host, there appears to be a gradual transition in emergence hole position from the cauda to a dorsal position just posterior to the thorax (Fig. 1H).

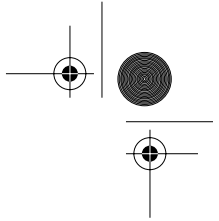
In summary, our results indicated that the phylogenetic tree inferred from parsimony analysis of the mitochondrial NADH1 and 16S rDNA genes was useful with respect to tracing the evolutionary transitions of various morphological, biological, and behavioural characters in Aphidiinae. Our trees are generally compatible with a smooth transition from one character state to another and in most cases, represent the most parsimonious solution. Some characters are evolutionarily more labile than others (e.g. egg shape, mandible shape, pupation behaviour). An examination of these characters in a broader range of taxa within Braconidae may provide insights into which characters are generally more labile and which are conserved.

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