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Grooming behaviours in the Hymenoptera (Insecta): potential phylogenetic significance

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Grooming behaviours from representatives of 36 families of Hymenoptera were video recorded and analysed. Thirty-three distinct types of grooming movements were recognized. The evolutionary pattern of each behaviour is discussed. Some behaviours displayed consistent variation between taxa, and appear to be informative regarding higher level relationships within the order. Putative synapomorphies are reported that support the monophyly of the Apocrita, Cynipoidea, Platygastroidea, Ichneumonoidea, and Chalcidoidea including the Mymaridae.

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INTRODUCTION

The phylogeny of the Hymenoptera has attracted increasing attention since the work of Königsmann (1976, 1977, 1978a, b), who proposed a partially resolved

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cladogram based on a large data set assembled from the literature. However, his analysis failed to provide evidence for relationships among many groups of the non-aculeate apocritan families. The second major series of studies are these of Rasnitsyn (1969, 1980, 1988). Rasnitsyn (1988) proposed a new classification based on the phylogeny generated from his data set which additionally incorporated information from the rich fossil record of the order. However, in his analysis he did not strictly follow cladistic principles, and therefore, his hypotheses have been subject to critiques. Despite this, subsequent studies have provided evidence that support several but by no means all of his proposed relationships. In particular, the concepts of sister-group relationships between the Orussidae and Apocrita (Gibson, 1985; Johnson, 1988; Whitfield, Johnson & Hamerski, 1989; Basibuyuk & Quicke, 1995; Vilhelmsen, 1996), between the Ichneumonoidea and Aculeata (Quicke, Fitton & Ingram, 1992; Sharkey & Wahl, 1992; Basibuyuk & Quicke, 1995) and the position of the Xyelidae as the most ancient extant family of the Hymenoptera (Gibson, 1993) have been supported by a growing body of evidence. Up to the present, only the aculeate hymenopteran clade has been more critically analysed (Brothers, 1975; Carpenter, 1986; Brothers & Carpenter, 1993) and the relationships among remaining hymenopteran groups are still far from satisfactorily resolved.

Our attempt here, has been to determine the grooming behaviour repertoire of representatives of as many hymenopteran families as possible, and to discuss each behavioural character in evolutionary terms. Possible correlations between the antennal grooming behaviour and the morphology of antennal cleaning apparatus (Basibuyuk & Quicke, 1995) were also investigated.

Use of behavioural characters as phylogenetic indicators

Behavioural characters that are effectively hard-wired, as are common in invertebrates, like any other genetically determined characters, are potentially useful for phylogenetic reconstruction (Jander, 1966; Farish, 1972; van den Assem, Gijswijt & Nübel, 1982; van den Assem, in den Bosch & Prooy, 1982). Furthermore, evidence suggests that behavioural characters are no more homoplasious than morphological or molecular ones (Prum, 1990; de Queiroz & Wimberger, 1993; Kurt & Hartl, 1995). However, the use of behavioural characters in phylogenetic analyses presents a number of difficulties that are not normally encountered in morpho-taxonomic studies. One such is the observer's inability to know whether the behavioural repertoire recorded for a given taxon is complete and whether it has been unduly influenced by the recording protocol. In addition, the problem of taxon sampling can be considerable as there are few groups where it would be possible to record behaviours for all taxa, and often it may only be possible to study one individual within a species (Farish, 1972). Behavioural studies therefore often involve a large measure of generalization about higher taxa from only a small sample of species (Valentine & Glorioso, 1978). Finally, there are potential difficulties with homologizing the character states for phylogenetic reconstruction (Prum, 1990; de Queiroz & Wimberger, 1993). Nevertheless, as the work on wasp nest architecture by Wenzel (1991) has shown, insect behaviour can provide a great deal of useful information for phylogenetic analysis, and therefore, as Alexander (1962: 53) put it, "The prime question now is not whether to use behaviour ..., but when and how to use (it)".

In insects, behaviour can be divided into two subcategories: (1) maintenance activities such as locomotion, grooming, and feeding, which keep an insect in good shape but usually have little influence on other individuals, and (2) communicatory activities which influence the moods and activities of conspecifics (Matthews & Matthews, 1978). Here we only deal with the former.

Potential of grooming behaviours in evolutionary studies

In a traditional sense, insect grooming movements are usually stereotyped (Wilson, 1962; Thelen & Farish, 1977; Turillazzi & Zapponi, 1982). In beetles, for example, individuals with missing or abnormal appendages attempt identical acts to those performed by normal individuals, even though actual grooming is impossible (Valentine, 1973). Similarly, in praying mantids, fore leg amputation does not effect the head grooming pattern (Zack, 1978a). However, some crickets with amputated fore legs occasionally use their middle legs or hind legs to clean the head which is normally a function of the fore leg (Honegger, Reif & Müller, 1979). A detailed comparative study on grooming behaviour of the wild and mutant strains of a braconid wasp, *Habrobracon hebetor* (Say), suggested that the grooming behaviour was highly fixed and controlled by central neurones. The main grooming behaviour repertoire was identical in mutant and wild-type strains, and amputation of appendages did not affect the fixed grooming motions. Environmental factors may change the frequency of a particular behaviour but cannot affect its nature (Thelen & Farish, 1977). Grooming behaviour can be induced by external stimuli or may occur in the absence of obvious external stimulus, or be exhibited as displacement activity, or as a consequence of social facilitation (Connolly, 1968; Waldorf, 1978).

Little is known about the sequential organization of grooming behaviour in insects. Several studies concerning flies (Dawkins & Dawkins, 1976; Walker & Archer, 1988), wasps (Thelen & Farish, 1977), mantids (Zack, 1978b, c), and crickets (Lefebvre, 1981) suggest that it can often form two clusters. The anterior cluster consists of grooming of antenna, head, mesosoma, fore legs and middle legs for all of which the fore legs are largely responsible, and the posterior cluster, which includes grooming of wings, metasoma, middle legs and hind legs, and which is mainly carried out by the hind legs.

Grooming behaviours vary from group to group in a way that provides valuable aids to classification and phylogeny (Jander, 1966; Farish, 1972; Valentine & Glorioso, 1978). Within the Insecta, there are several comparative studies dealing with variation at various taxonomic levels. Szymanski (1918) and Jander (1966) used grooming to make inferences about relationships at order level. Others have made comparisons at family group level (Heinz, 1949; Gangwere, 1958; Farish, 1972; Valentine, 1973; Jander, 1976; Valentine & Glorioso, 1978). Farish (1969, 1972) carried out the only comparative study on grooming and superfamily level relationships within the Hymenoptera. In total, he investigated the grooming behaviour of 115 species across the order, but uneven taxon sampling meant that several major groups were not examined at all. Additional information of grooming behaviour in particular taxa, especially for ants and bees, have been provided in a number of smaller, more or less isolated investigations: Shuckard (1866), McCook (1879), Schulze (1924), Beeken (1934), Jung (1937), Weber (1958), Olberg (1959), Heatwole, Davis, & Wenner (1962), Wallis (1962), Wilson (1962, 1979), Batra (1964),

Sudd (1967), Kitano (1976), Jander (1976), Thelen & Farish (1977), Jander & Jander (1978), Michener, Winston & Jander (1978), Cane *et al.* (1983), Conway (1983), Kimsey (1984), Schönitzer & Renner (1984), Blom & Velthuis (1988), Lee & Lee (1989), Beani & Calloni (1991), Callow (1993), and Oi & Pereira (1993).

METHODS

One hundred and eight species, collectively representing 17 of the 20 currently recognized superfamilies of Hymenoptera, were observed. Voucher specimens are deposited at The Natural History Museum, London. Both field-collected and cultured specimens were videorecorded using a camera attached to a binocular microscope and low intensity cool fibre-optic illumination. Minimum recording time of grooming behaviour was 15 minutes, but many individuals were recorded for much longer periods up to two hours. In many cases, wasps would groom spontaneously when left undisturbed; however, in some cases, grooming movements had to be induced. This was achieved either by bringing specimens out of a fridge (6°C) after a period of 5–10 minutes cool treatment, or by dusting them with pollen or polyporus spores. Video tapes were analysed using a Panasonic, NV-J45B video player with variable speed and frame by frame playback.

MacClade (Maddison & Maddison, 1992) was used to examine character state changes on cladograms. Chi-square (χ^2) and Spearman rank correlation coefficient (r_s) tests were applied to search for possible associations between the morphology of antenna cleaner and the antennal grooming behaviour (Siegel, 1956).

Definition of grooming behaviours in the hymenopteran repertoire mainly follows Farish (1972). Intraspecific or interspecific variation in a particular grooming behaviour within higher taxa was not considered in constructing the family level data matrix. Data from all members of cynipoids and a large part of chalcidoids were combined at superfamily level, though those for Mymaridae are given separately because this family has been shown to be the sister group of Chalcidoidea (Gibson, 1986). Absence and presence of a particular behaviour are represented by 0 and 1, respectively, and are not intended to infer any character state polarity. Family arrangement follows Gauld & Bolton (1988), except the Platygastroidea is treated as separate, a superfamily in accordance with Goulet & Huber (1993).

TERMINOLOGY AND TAXA OBSERVED

The terminology used to describe various grooming behaviours differs from one author to another and each term may bear a functional implication. Some authors use the term 'cleaning' only if the mouthparts are used for this purpose (Valentine, 1973). Use of legs was therefore, referred to as 'rubbing' by some workers to distinguish it from cleaning (Valentine, 1973,1986; Valentine & Glorioso, 1978; Smith & Valentine, 1985). In this study, we use the term 'grooming' to apply to all actions that serve any of the following four functions: (i) cleaning (Valentine, 1973; Honegger, Reif & Müller, 1979); (ii) transferring a substrate from one body part to an other (Jander, 1976; Wilson, 1979; Cane *et al.*, 1983; Kimsey, 1984; Kovac & Maschwitz, 1989; Beani & Calloni, 1991; Rakitov, 1992); (iii) removal of parasites

and pathogens from the body (Boecking, Rath & Drescher, 1993; Dustmann, 1993; Oi & Pereira, 1993); and (iv) rearrangement of body parts relative to one another (Waldorf, 1978). Our results pertain entirely to self-grooming, and we do not consider mutual grooming as shown by some social Hymenoptera.

For clarity and consistency we have followed Jander's (1976) classification of grooming behaviour into rubbing, scraping, and nibbling. (1) Rubbing behaviour, involves two body parts stroking each other back and forth without losing close contact throughout the action. Both body parts may be moved simultaneously or one may stroke the other, the latter remaining stationary. Even though during rubbing, the effective movements are bidirectional, dirt is gradually pushed out distally due to the nearly uniform orientation of spines and setae on the structures involved. Rubbing is a terminal cleaning movement insofar as it eliminates dirt from some body area rather than transferring it from one area to another. (2) In scraping, the effective action is only in one direction. The parts doing the cleaning touch one restricted area to be cleaned and stroke along it. A return stroke without physical contact may follow, if the scraping action is to be repeated. Scraping transfers dirt or substrate from the cleaned to the cleaning part. (3) Nibbling is a cleaning movement in which an antenna or leg is taken into the inner mouthparts and then pulled through them.

Appendix 1 lists material observed with the full grooming repertoire recorded for each species.

RESULTS AND DISCUSSION

We have classified the grooming behaviour repertoire according to what part is being groomed. For nearly all behaviours observed, it is clear that only one part is being groomed at a particular time. However, in a few instances, more complex behaviours involving simultaneous cleaning of two or more body parts occur and these have been treated separately (behaviours 31–33). These behaviours are distinct from occasions in which two distinct grooming behaviours are being carried out simultaneously. For example, nibbling of a fore leg by mouthparts (behaviour 19) or grooming of head by fore legs (behaviour 6) are sometimes, though infrequently, carried out at the same time as the hind legs are being used to groom the metasoma (behaviour 11). Similarly, behaviours 19 and 24, behaviours 19 and 30, behaviours 6 and 27, behaviours 2 and 6, behaviours 1 and 11, behaviours 7 and 29, and behaviours 24 and 30 may occur at the same time, but both behaviours are also displayed independently by the same wasp, usually more often than in combination. Complex grooming always involves a single scraping structure and two or more parts being scraped in a complex but relatively stereotyped movement pattern.

To make our survey of grooming behaviour in the Hymenoptera and related orders as complete as possible, we have incorporated data from a range of literature sources in addition to our own observations. The sole comparative study was by Farish (1972), and we accept and use his data either for a whole taxon when we have been unable to examine any representatives of that group, or in some cases to supplement our observations when it is clear that we have seen only a part of that taxon's total grooming repertoire. Grooming behaviours displayed for the hymenopteran families or superfamilies studied are summarized in Appendix 2.

Hymenopteran grooming behaviours and their evolutionary patterns

Antenna scraping (Behaviours 1–5)

Nibbling is part of the cleaning repertoire of most ancient insects as a main cleaning movement (Valentine & Glorioso, 1978), and it has been suggested that it ought to be regarded the most plesiomorphic condition for the tracheate arthropods (U. Jander, 1966; R. Jander, 1976). Valentine & Glorioso (1978) argued that all primitive arthropods also have 'rubbing' (pedal cleaning) movements, but in most cases it is impossible to decide objectively which one came first.

Within the Hexapoda, the most plesiomorphic pattern of antenna cleaning appears to be unassisted-oral antenna cleaning in which the antenna is cleaned by the inner mouthparts and the legs are not involved. The antenna bends down into the mouth (sometimes with the help of its intrinsic musculature). This occurs in the Collembola (*Sinella coeca*) (Waldorf, 1978), Diplura (Valentine & Glorioso, 1978), Thysanura (Jander, 1966), Embioptera, Psocoptera (Valentine, 1986), Blattodea (Blaberidae) (Smith & Valentine, 1985) and Coleoptera (Cantharidae) (Valentine, 1973).

The next step in the evolution of antenna cleaning may be leg-assisted oral cleaning, of which at least three different types may be distinguished. (1) Cleaning of antenna assisted by ipsilateral fore leg: the ipsilateral fore leg pulls the antennae into the mouth. This mode of behaviour is present in the Thysanura, Diplura, Orthoptera, Dermaptera, Blattodea, Mantodea, Zoraptera, planipennian Neuroptera and Coleoptera (Gangwere, 1958; Jander, 1966; Valentine, 1986). A variant of this behaviour occurs in some Coleoptera (Melyridae); both antennae may be passed simultaneously through the mouth with the help of one fore leg (Valentine, 1973). (2) Antenna cleaning is assisted by contralateral fore leg: contralateral fore leg pulls the antenna into the mouth. This occurs in the Blattodea (Cryptocercidae, Blattidae and Blattellidae) (Jander, 1966; Smith & Valentine, 1985) and Coleoptera (Rhynchitidae) (Valentine, 1973). (3) Antenna cleaning assisted by both fore legs: one antenna at a time is pulled by both fore legs into the mouth. This mode was found in the Blattodea (*Periplaneta brunnea*) (Smith & Valentine, 1985). There is no convincing evidence concerning the sequence in which these three modes evolved.

Cleaning of the antenna as well as of other appendages and body parts by the legs (pedal grooming) is, therefore, postulated as an apomorphic state. Both states are displayed by some Orthoptera (Lefebvre, 1981) and Coleoptera (Valentine, 1973). As might be expected, cleaning the antenna by the fore legs, which may have a specialized cleaning apparatus, involves almost the same initial stages as that of assisted-oral cleaning.

Antennal grooming in the Hymenoptera is always a scraping movement and involves the specialized basitarsus-fore tibial spur complex of the fore leg termed the antenna cleaner (Schönitzer & Lawitzsky, 1987; Basibuyuk & Quicke, 1994, 1995). The spur is usually curved, and in the case of the Apocrita, the basitarsus is distinctly notched (Fig. 1A). This structure is used to grip and scrape the antenna even in those taxa in which the spur and basitarsus are not so modified (Fig. 1B). The antenna is almost always drawn between the spur and basitarsus from base to apex (Fig. 1C).

Behaviour 1. Single antenna scraping by ipsilateral fore leg. One antenna is groomed at a time by the antenna cleaner of the ipsilateral fore leg and the different antennae are usually groomed consecutively (Fig. 2A). Ipsilateral antenna cleaning (behaviour

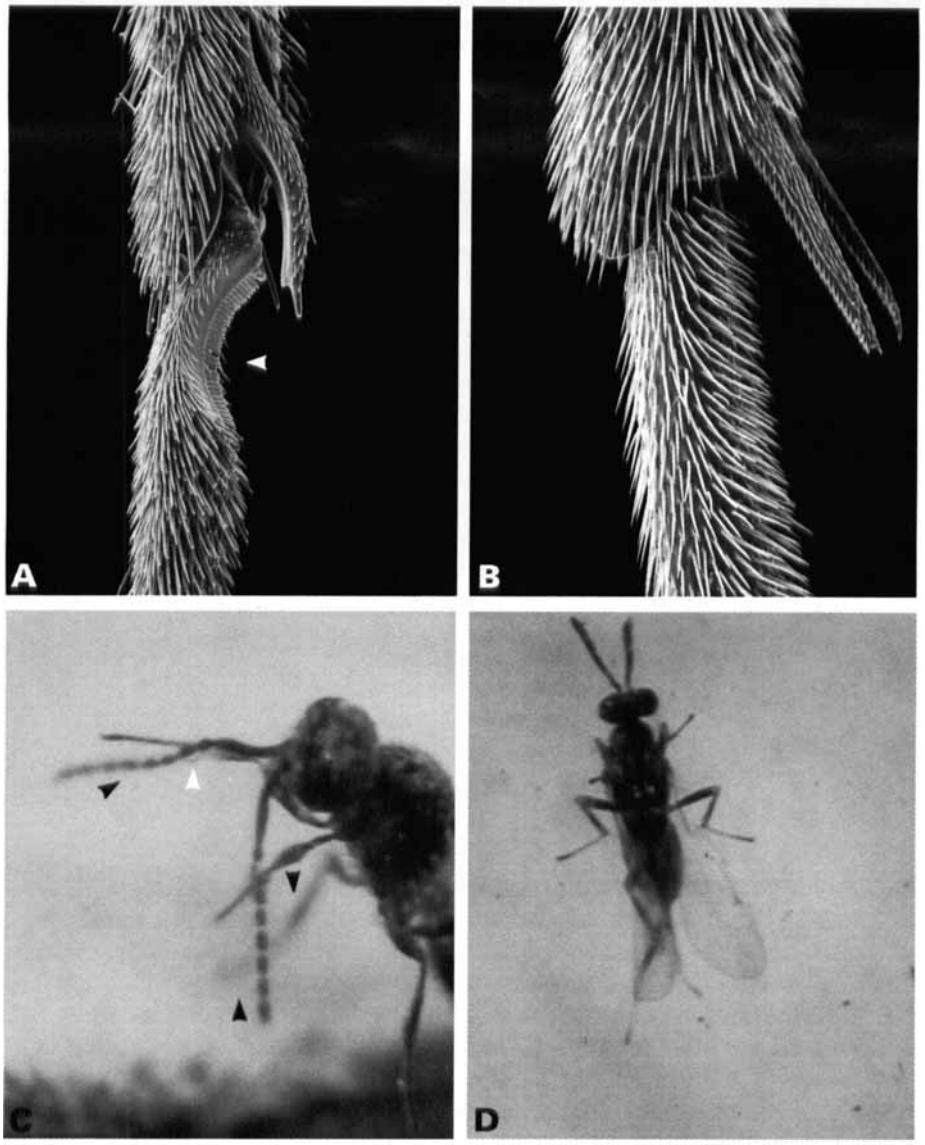


Figure 1. Antenna cleaner (A, B) and grooming behaviour (C, D). A, *Aulacus striatus* Jurine (Aulacidae), basitarsal notch (arrow); B, *Arge cyanocrocea* (Forster) (Argidae); C, gen. sp. (Platygastridae) double antenna grooming, fore tibial spur (white arrow); D, *Torymus* sp. (Torymidae) chalcid type wing grooming.

1) seems likely to be evolved from ipsilateral assisted-oral antenna nibbling, in which the ipsilateral leg initially just served to grasp and pull the antennae down into the mouth. Over time, it may have lost this function, and instead gained the running motion of the fore leg along the antenna from base to apex. This grooming behaviour occurs in the Orthoptera (Acrididae) (Gangwere, 1958), Coleoptera (Jander, 1966; Valentine, 1973), Neuroptera (Jander, 1966; Kovarik, Burke & Agnew, 1991), Lepidoptera (Jander, 1966; Barry & Nielsen, 1984), and Hymenoptera (Farish,

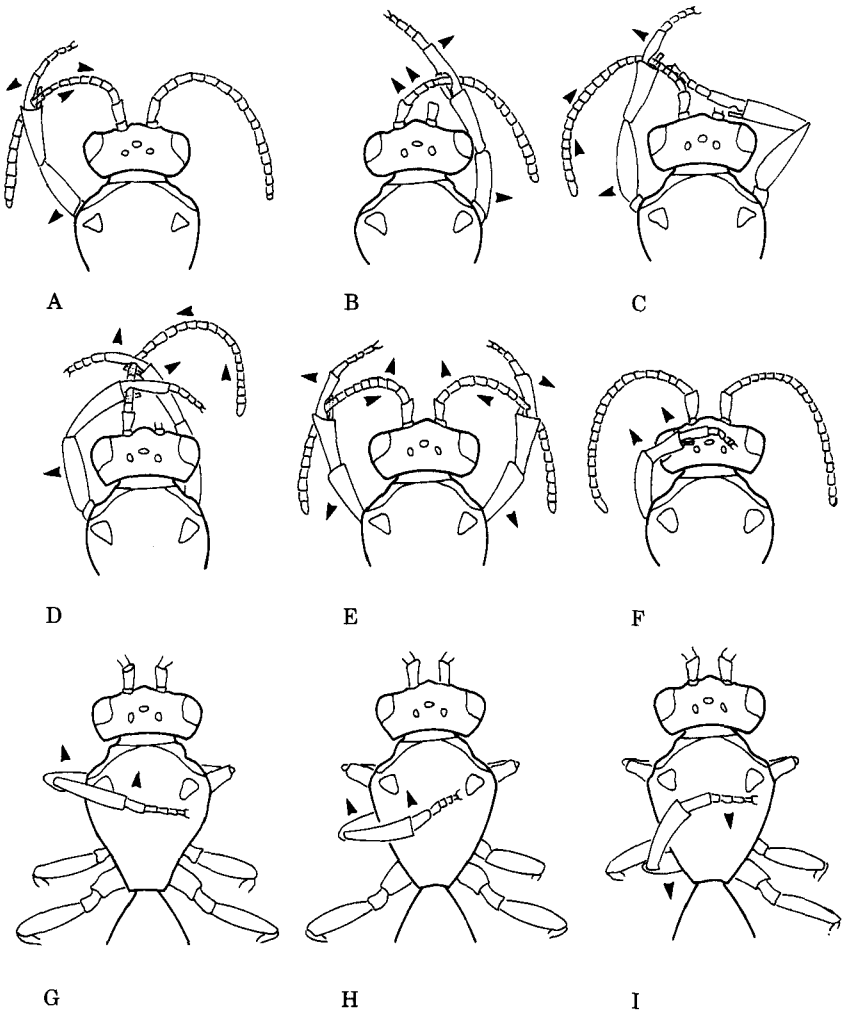


Figure 2. Illustrations of antennae (A–E) and body grooming. A, ipsilateral (behaviour 1); B, contralateral (behaviour 2); C, one antenna with one antenna cleaner (behaviour 3); D, one antenna with both antenna cleaners (behaviour 4); E, double (behaviour 5); F, head with fore leg (behaviour 6); G, thorax with fore leg (behaviour 7); H, thorax with middle leg (behaviour 8); I, thorax with hind leg (behaviour 9).

1972). Although, the antenna is scraped by the ipsilateral fore leg in the above mentioned taxa, each taxon may clean the antenna in a different manner. In addition, only the basal part of the antenna, where the mouth is unable to reach, is rubbed by ipsilateral fore leg in the Diplura and Zoraptera.

In the Hymenoptera, behaviour 1 occurs across the whole order as well as in many other insect orders, and it is therefore suggested that this represents the plesiomorphic condition. However, from the present observations it appears to be absent from the Xyelidae, Argidae, Pergidae, and Chalcidoidea. Surprisingly, this is the only common and constant antenna cleaning movement in bees (Jander, 1976; Kimsey, 1984; Schönitzer & Renner, 1984; Naumann, 1991), in which one

might expect novel behavioural patterns. Some mymarids and cynipoid wasps also lack this grooming movement, but this could either be due to insufficient recording or intrafamilial variation. Farish (1972) reported that cynipoids do not show this behaviour, but he only observed one species of figitid and we have observed it in the ibaliid and several cynipids and charipids investigated (Appendix 1). Absence of this behaviour is postulated as a putative synapomorphy for the Chalcidoidea since no members of that superfamily (though excluding mymarids) have been observed to perform this behaviour.

Behaviour 2. Contralateral antenna scraping. One antenna is groomed at a time by the antenna cleaner of the fore leg on the opposite side of the body (Fig. 2B). The same argument as presented for behaviour I can be applied here and to other patterns of antenna cleaning. Thus, behaviour 2 may have evolved from the contralateral leg-assisted oral cleaning. It is present in the Neuroptera, Coleoptera (Valentine, 1973) and Hymenoptera. Within the Hymenoptera, it is absent in almost all aculeate Hymenoptera with the exception of a few multillid wasps; nor have any xiphydriid (Farish, 1972), xyelid, argid, cimbicid, diprionid, pergid, siricid, orussid, proctotrupid, megaspilid and chalcidoid wasps been observed to perform this behaviour pattern. Unfortunately, in the case of several of these taxa, it is not possible to be absolutely certain about its absence due to possibly incomplete repertoires recorded, but the last two (megaspilids and chalcidoids) are represented in our data set by sufficient observation and large repertoires; we are, therefore, reasonably confident that both groups lack this behaviour.

Behaviour 3. Simultaneous grooming of one antenna by both legs but with the antenna cleaner of only the ipsilateral fore leg being involved. The ipsilateral fore leg grips the antenna, the basitarsus (and sometimes the rest of the tarsus) of the contralateral fore leg helping to hold the antenna in place (Fig. 2C). In this paper behaviours 3 and 4 are evaluated separately; they were not distinguished from one another by Farish (1972) who referred to both as 'chalcid type'. Both behaviours appear to be derived from two leg assisted-oral antenna cleaning; behaviour 3 could be interpreted as the primitive state that give rise to behaviour 4. They appear to be performed by some Hemiptera (Hlavac, 1975), Coleoptera (Valentine, 1973), Neuroptera (Jander, 1966), Diptera (Walker & Archer, 1988) as well as by some Hymenoptera. In the Hymenoptera, behaviour 3 is only displayed by members of the symphytan families Pamphiliidae, Argidae, Diprionidae, Pergidae and Tenthredinidae. No apocritan families were found to routinely display this behaviour although, one ichneumonid species (*Odontocolon* sp.) performed this action on a few occasions.

Behaviour 4. Simultaneous grooming of one antenna with antenna cleaners of both ipsi-and contralateral fore legs. One antenna at a time is scraped simultaneously by the antenna cleaner of both for legs (Fig. 2D). Behaviour 4 is exhibited by three symphytan families, the Argidae, Cimbicidae and Pergidae. It is a prominent pattern of the repertoire of the Megaspilidae (Ceraphronoidea), Chalcidoidea, Mymaridae, Cynipoidea and Proctotrupidae, and some members of Platygastroidea (see Appendix 1 for exceptions). According to Farish (1972), Xiphydriidae, Braconidae, Ichneumonidae, Figitidae, Chalcidoidea, Diapriidae, Heloridea and Evaniidae show 'chalcid type' antenna grooming behaviour, but as mentioned above, he did not distinguish between behaviours 3 and 4. In our present extensive observations,

almost all members of either the Braconidae or Ichneumonidae (Ichneumonoidea) were found not to exhibit either behaviour, and therefore we treat these results of Farish with some scepticism.

Behaviour 5. Double antenna scraping. The antenna cleaner of both fore legs are passed distad along their respective ipsilateral antenna (Fig. 1C, 2E). This behaviour is only known to occur in some Coleoptera (Valentine, 1973) and Hymenoptera and is presumably independently acquired in these two orders. There is no nibbling equivalent of this behaviour and so we postulate that it arose from ipsilateral antenna grooming (behaviour 1). Behaviour 5 is carried out by most hymenopteran taxa, although no members of the symphytan families Argidae, Diprionidae, Pergidae and Tenthredinidae and parasitic taxa chalcidoids, megaspilids, pelecinids or proctotrupids displayed it. It was also apparently absent from the repertoires of some species of the Cynipoidea and Ichneumonoidea, though it was present in others. Our sampling for proctotrupids was rather limited and so we cannot claim that it definitely does not occur in these. It should be noted that this behaviour is rare in bees (Jander, 1976; Kimsey, 1984). Considering its distribution, this behaviour may be plesiomorphic for the Hymenoptera and its absence in some families of the tenthredinoid lineage (except Cimbicidae), Chalcidoidea and Megaspilidae, (and also Pelecinidae and Proctotrupidae if the present results are substantiated) due to secondary loss.

Body grooming (Behaviours 6–11)

Grooming of the whole body (except the pronotum and head) by mouthparts can be considered as the plesiomorphic behaviour in the Insecta, though in the Collembola the lower part of head is rubbed by the everted vesicles of the collophore (Waldorf, 1978). As it is physically impossible to clean the head and pronotum using the mouthparts, these always have to be groomed by the fore leg and the wide distribution of this action renders it phylogenetically uninformative. Oral grooming of the body is commonly displayed by the Thysanura, Microcoryphia, Diplura, Collembola, Dermaptera, Embioptera, Zoraptera and members of the orthopteroid complex, including Blattaria, Isoptera, Mantodea and Orthoptera. Many of the above orders also have one or more leg grooming movement. Oral grooming become gradually replaced by leg grooming in the holometabolous insects.

Body grooming in the Hymenoptera is a scraping movement and involves the inner (ventral) surface of the tibia and, to a lesser extent, the tarsus of the legs. All parts of the body are cleaned by various legs and depending upon function, each leg may have various structures, such as brushes, combs and modified seta, for this purpose (Hlavac, 1975; Jander, 1976; Mason, 1976; Kimsey, 1984; Schönitzer & Lawitzky, 1987; Basibuyuk & Quicke, 1995).

Behaviour 6. Head scraping by fore leg(s). Any part of head and eyes being groomed by the fore leg(s) in various actions (Fig. 2F). With one exception, the head is always scraped using a fore leg. The exception, noticed by Jander (1976), is the bumblebee, *Bumbus americanorum* (Fabricius), in which the middle leg is used. Head grooming has not been recorded in the Diprionidae, Pergidae, Dryinidae or Sapygidae and this is probably due to failure to observe a complete repertoire rather than any loss. It is an underived condition for Hymenoptera.

Behaviour 7. Thorax scraping by fore leg(s). Pro- and mesothorax are scraped by foretarsi (Fig. 2G). The fore legs are also responsible for cleaning the thorax in some

taxa, but this was not observed in any Symphyta, Cynipoidea (except *Ibalia*), Proctotrupoidea (except *Helorus*), Evaniidae, Dryinidae and Sapygidae. Further, it appears to be absent in many bees (Jander, 1976; Kimsey, 1984). Although, this behaviour occurs in many other insect orders, complete lack of it in all symphytan families investigated suggests that it is not part of the hymenopteran groundplan, and therefore it can be considered as an apomorphic condition.

Behaviour 8. Thorax scraping by middle legs. The pleura and dorsum may be groomed by the midtarsi (Fig. 2H). In contrast to behaviour 7, this behaviour is performed by many bees and sphecid wasps (Jander, 1976; Kimsey, 1984; Farish, 1972), the Tiphiidae and Chrysidoidea (the Dryinidae, Bethylidae and Chrysididae). Use of middle legs seems to be a clear replacement for the fore legs in the case of bees. It has been recorded for only one parasitic wasp family in the present study, viz. the Megaspilidae. It may be considered as an apomorphy for the Aculeata with respect to non-aculeate Hymenoptera.

Behaviour 9. Thorax scraping by hind legs: The pleura and dorsum may groomed by the hindtarsi (Farish, 1972) (Fig. 2I). This behaviour was not observed in the present study but has been reported for the Evaniidae (Farish, 1972) and one member of the Apidae (Jander, 1976); it is therefore probably a derived, albeit in the present context phylogenetically uninformative, condition.

Behaviour 10. Pleura groomed by the side of the fore femora and tibia. The leg is reflected back and bent at the femoral-tibial joint during grooming (Farish, 1972) (Fig. 3A). This behaviour occurs widely throughout the order, and was also reported in the Formicidae and Chrysidoidea by Farish (1972). Absence of this behaviour in the repertoire of the basal symphytan families suggests that it is a derived condition within the order.

Behaviour 11. Metasoma and ovipositor scraping by hind leg. One, or more often, both hind legs scrape the dorsal and lateral surfaces of metasoma. The ventral surface of the metasoma is often also groomed in this action. In female insects, the ovipositor is often scraped as part of a bout of metasoma grooming (Fig. 3B). Cleaning of the metasoma by the hind leg occurs in all taxa in the Hymenoptera, with the exception of the Cynipoidea of which members of three families, the Cynipidae, Charipidae and Ibaliidae, were included in this study. However, Farish (1972) also observed this in one species of Figitidae. This behaviour also occurs in many other insect orders and is therefore probably plesiomorphic within the Hymenoptera.

Wing scraping (Behaviours 12–18)

The earliest winged insects, the Palaeoptera, of which only the Ephemeroptera and Odonata survive, have wings that are much too large to be cleaned by their relatively short legs. No wing cleaning movements are known in the Ephemeroptera, but in the Odonata, wing cleaning movements involving the abdomen have been described for members of both the Zygoptera and Anisoptera (Jander & Jander, 1978; Utzeri, Falchetti & Raffi, 1987). In the Blattodea, the wings are similarly mainly cleaned by the abdomen but some members of the group use their hind legs to scrape the upper surface of the resting wings (Smith & Valentine, 1985). The Mantodea clean the wings by shaking them in a raised position (Zack, 1978c).

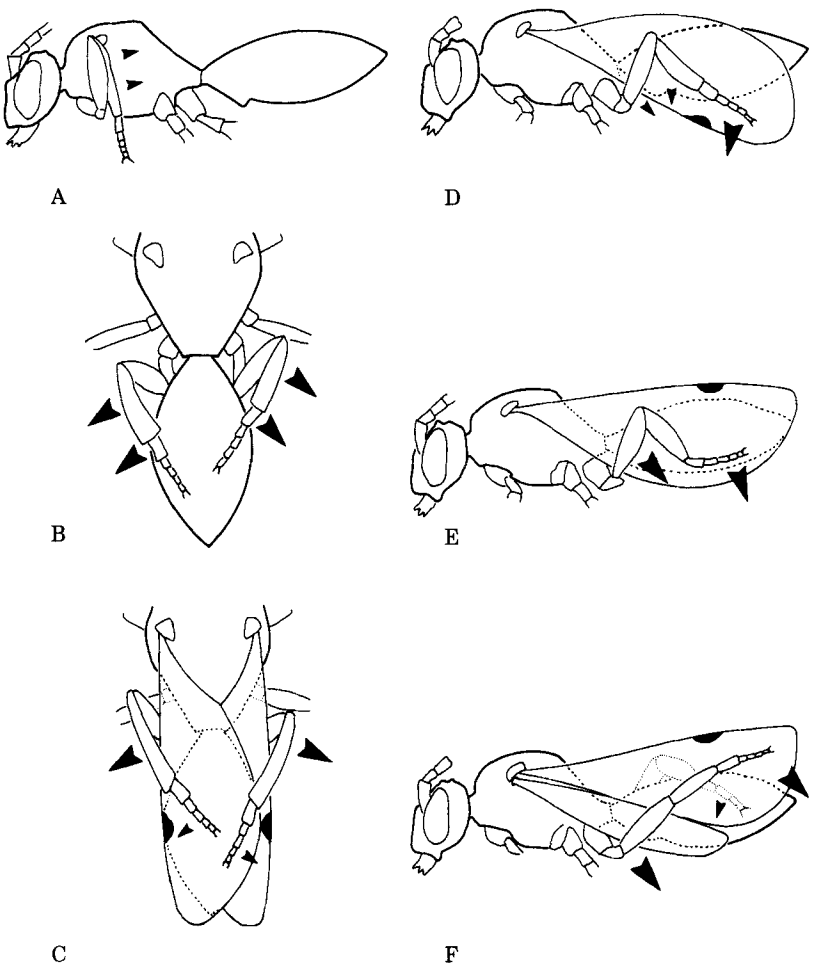


Figure 3. Body (A, B) and wing (C–F) grooming. A, pleura by fore leg (behaviour 10); B, metasoma by hind legs (behaviour 11); C, dorsal surface of the resting fore wings by hind legs (behaviour 12); D, dorsal surface of the raised wings by hind legs (behaviour 13); E, ventral surface of the raised wings by hind legs (behaviour 14); F, chalcid type wing grooming (15).

Another 'orthopteroid' group, the crickets (Orthoptera) has no assisted wing cleaning, instead they rub their raised wings against each other (Lefebvre, 1981). Among those Neoptera that have large wings with primitive venation, it is within the Megaloptera (here taken to include the Rhaphidiina) that abdominal wing cleaning still plays an important role (Jander, 1966). The snakefly, *Raphidia bicolor* Albarda (Raphidiidae) displays periodical movements of the abdomen which are called abdominal stretching, usually considered as a part of the courtship ritual, but it has also been noted in non-courting snakeflies of both sexes (Kovaric *et al.*, 1991). When the insect is engaged in this behaviour, it secretes a chemical substance from the apex of the abdomen, then with a rapid vibration the abdomen traces an are across the under-surfaces of both pairs of slightly elevated wings from stigma to stigma. This motion is similar to the abdominal wing cleaning of the Palaeoptera, but

whether the behaviour in *Raphidia* is primarily concerned with cleaning is unclear, and similarly it is not possible to determine whether if it is evolved from, or gave rise to, the courtship movements.

Jander & Jander (1978) suggested that scraping the dorsal surface of the resting fore wing by the hind legs is the most widespread and simplest novel wing cleaning movement among the Neoptera; therefore, this cleaning movement was considered to be one of the earliest apomorphic wing cleaning movements to evolve within that group. They also provided some evidence to support this hypothesis as well as to explain that this novel behaviour originated from the most common locomotory function of the hind legs. This movement is also shown by cockroaches and some Orthoptera.

In the Coleoptera, cleaning of wings may achieved in several different ways. In addition to raising and lowering of the elytra, extending the wings with or without abdominal assistance and unfolding and refolding of the wings, they can also use the hind legs for cleaning both the elytra and the wings. Scraping the dorsal surface of the elytron (fore wing) in resting position by the hind legs is displayed by most beetles. In addition some beetles show one of two further behaviours. One of these is raising the elytron to enable placement of the hind legs on the dorsal surface of hind wings to perform scraping movement, and the other is lifting of the hind wing during the abdominal scraping by hind legs, which may give an opportunity for cleaning the under-surface of hind wings (Valentine, 1973). Compared with the above groups, many Diptera, Mecoptera and Hymenoptera display several derived wing cleaning behaviours.

In the Hymenoptera in general, wings are only groomed by the hind legs. Farish (1972) recognized three types of wing grooming movement for the Hymenoptera whereas Jander & Jander (1978) distinguished seven types, as in bees. In this paper we define seven types. Some of Jander & Jander's movements ("scraping the upper surface of the fore wing ... hind wing" and "... under surface of the fore wing ... hind wing") are not distinguished here because they are simply parts of particular grooming bouts. However, we have added one novel movement which is performed by ichneumonoid wasps (behaviour 16). Jander & Jander (1978) suggested that there are two ancestral wing cleaning movements in the Hymenoptera and that these had persisted unchanged throughout the phylogenetic radiation of the order (behaviours 12 and 18).

Behaviour 12. Scraping the dorsal surface of the resting fore wings. While all the wings are in their resting position, one or both hind legs scrape (mainly the distal part of the resting fore wings) with their tarsi (Fig. 3C). This behaviour appears to be part of the groundplan for Hymenoptera as suggested by Jander & Jander (1978). It is performed by members of all symphytan families (except perhaps Siricidae) as well as many families of parasitic Apocrita. It is also exhibited, though only rarely, by some Apoidea (Jander & Jander, 1978). According to Farish (1972), it is only displayed by symphytan families and two parasitic wasp families, the Ichneumonidae and Braconidae. However, we have also recorded this behaviour in most other parasitic families with the exceptions of the Heloridae, Pelecinidae, Gasteruptiidae and Megaspilidae. Absence of this behaviour may be regarded as a derived condition for Aculeata with respect to the rest of the Hymenoptera, and therefore its occurrence in the Apoidea (both Apidae and Sphecidae) is most parsimoniously considered as a secondary independent gain.

Behaviour 13. Scraping the dorsal surface of the raised wings. One or both pairs of wings bend down on the ventro-lateral surface of the metasoma, with the costal margin oriented downwards, then one or both hind legs simultaneously scrape the dorsal (upper) surface of fore wing, with a backward-downward motion, using the tibia and tarsi. After scraping, the fore wings return to a horizontal position, though with more distance from the side than the normal resting position. The hind wings remain between the hind legs and metasoma and are then scraped as above (Fig. 3D). It appears to be derived from behaviour 12 by lifting and lowering the wing to the side of the metasoma. This behaviour was observed in the Xyelidae, Pamphiliidae, Tenthredinidae and Orussidae and also in most parasitic and aculeate families, and it therefore appears to be part of the hymenopteran grooming groundplan.

Behaviour 14. Scraping the ventral surface of the raised wings. The scraping action and main movement of the wings and metasoma are the same as in behaviour 13. The positioning of the wings, however, are different. This time, the costal edges of the wings are oriented upwards and the ventral (underside) of hind wings are scraped by the hind legs (Fig. 3E). It is likely to be derived from cleaning of movement of the metasoma. While the metasoma is cleaned by the hind legs, the wings have to be raised to give access for the hind legs. During the period of the metasomal cleaning, the hindlegs may rub the wings from underneath. By taking one step further, the wings are twisted on their longitudinal axes so that their undersides now face outwards. The motions of hind legs are almost the same, as those involved in metasoma cleaning. This behaviour is not displayed by the Xyelidae, Pamphiliidae, Cimbicidae, Diprionidae, Pergidae, Xiphydriidae, Siricidae, Megaspilidae, most Chalcidoidea, Vespoidea (*sensu* Farish) and Formicidae. However, we have observed this in the Sapygidae. Absence of this behaviour in the repertoire of most symphytan families suggests that it is a derived condition for the Hymenoptera.

Behaviour 15. 'Chalcid type' wing grooming: a pair of raised wings of one side are groomed simultaneously between both hind legs on both surfaces of the wings, with costal margins either oriented upwards or downward. This is an extremely specialized action for cleaning the wings and is characteristic of the Chalcidoidea (Farish, 1972). In this, only one pair of wings is cleaned at a time by being sandwiched between both hind legs which then scrape both surfaces distally (Figs 1D, 3F). In addition to the Chalcidoidea it is also performed by some Cynipoidea and there is an indication that some members of the Xyelidae, Evaniidae, Gasteruptiidae, Proctotrupidae and Pompilidae may perform similar but incomplete movements. This behaviour has also been reported for *Drosophila* (Szebenyi, 1969), although it is more parsimonious to assume its independent derivation within Hymenoptera since most taxa including symphytan families do not perform this behaviour.

Behaviour 16. Ichneumonid type wing grooming: both pairs of raised wings are groomed simultaneously between tibia and tarsi of each respective ipsilateral hind leg (Fig. 4A). It is displayed by some ichneumonid, braconid and evaniid species, in a complete form, and some male Mutillidae display a similar action. It appears to be a synapomorphy for the Ichneumonoidea.

Behaviour 17. Scraping the base of the resting fore wing. When the middle leg scrapes the dorsal surface of the thorax, it sometimes reaches the base of the fore wing and scrapes it (Jander & Jander, 1978) (Fig. 4B). This behaviour is known to be

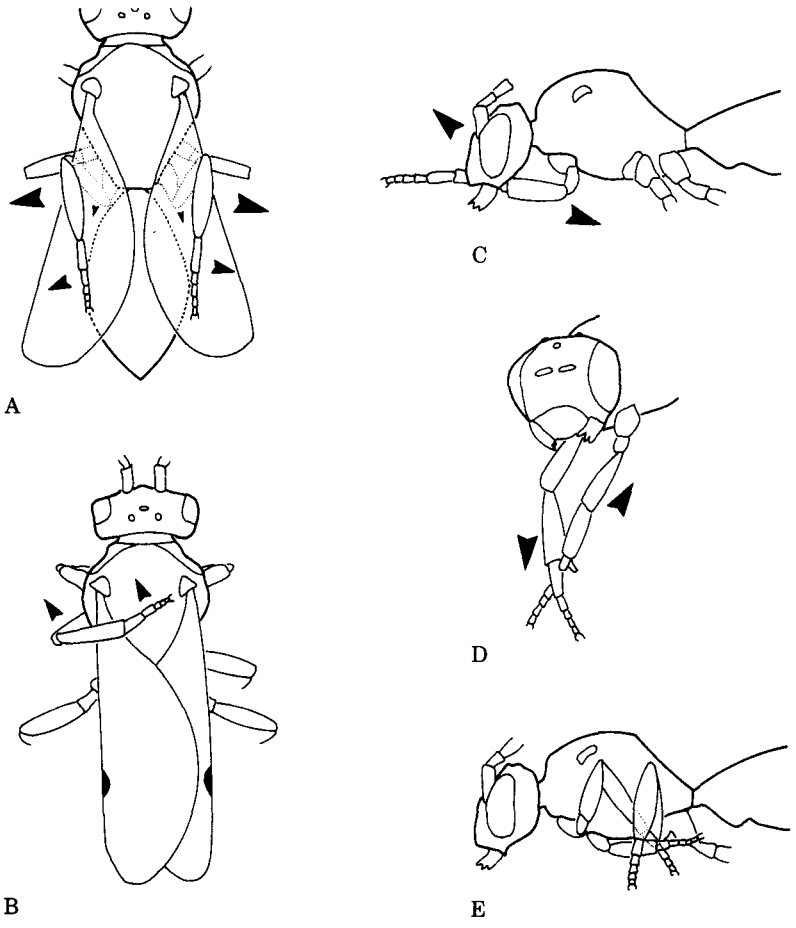


Figure 4. Wing (A, B) and leg (C–E) grooming. A, ichneumonoid type wing grooming (behaviour 16); B, base of fore wing by middle leg (behaviour 17); C, fore leg nibbling (behaviour 19); D, fore leg–fore leg rubbing (behaviour 20); E, fore leg–fore leg–middle leg rubbing (behaviour 21).

performed only by members of Apoidea (Jander & Jander, 1978), therefore it appears to be a synapomorphy for that group.

Behaviour 18. Fluttering. In this cleaning movement only the wings themselves are involved (Jander & Jander, 1978). It was only known to be performed by cephid sawflies (Cephoidea) and bees (Jander & Jander, 1978). In addition to observing this in another cephid, *Calameuta*, we also observed it in *Tremex* (Siricidae), *Atanycolus* (Braconidae) and *Epyris* (Bethylidae). Given this patchy distribution, it is questionable whether this can be considered plesiomorphic for the entire Hymenoptera. Rather, it seems far more likely (and it is more parsimonious) that its distribution represents several independent acquisitions.

Leg/appendage grooming (Behaviours 19–30)

As discussed above, the cleaning of any part of the body or appendages by the mouthparts is considered as a primitive state for the Insecta. Using mouthparts for

cleaning the appendages is dominant in the Thysanura, Diplura, Collembola, Zoraptera, Embioptera, Blattaria, Mantodea and Orthoptera. The grooming repertoire of Diplura, Embioptera, Zoraptera and Mantodea only includes the cleaning of appendages by mouthparts; no appendage-rubbing movements have been observed (Rita & Ananthasubramanian, 1991; Valentine, 1986; Valentine & Glorioso, 1978; Zack, 1978c). Similarly, only the mouthparts are used for the cleaning of appendages in the Collembola (Waldorf, 1978). Within the hemimetabolous insects, Zygoptera (Utzeri *et al.*, 1987), Blattaria (Smith & Valentine, 1978) and crickets (Lefebvre, 1981), may exercise both oral and pedal grooming for appendage grooming. The only holometabolous insect order which has both grooming movements—either oral cleaning or using the legs to groom each other—is the Coleoptera. Except for cleaning of the fore legs, the Diptera and Hymenoptera only use pedal grooming for this purpose. In general, oral grooming has been lost when the pedal grooming has evolved.

Fore legs are important tools for the grooming of antennae as well as other body parts, and are the only appendages that are groomed by the mouthparts. During grooming the grooming structures may accumulate dirt particles and it is important that they are removed, otherwise the efficiency of grooming may be compromised. In this respect, elimination of material from the fore legs by the mouthparts may be particularly significant.

Apart from the fore legs, all the other legs are rubbed against each other, even though detritus is concentrated on only one surface of a cleaning organ. It is not possible to hypothesize the evolution of any of steps leading to each behavioral pattern, although some hints may come from the oral grooming of the legs. In the Diplura, besides displaying single fore leg, middle leg, and hind leg grooming by mouthparts, some additional combinations have been observed. Fore leg–middle leg, fore leg–hind leg, and middle leg–hind leg of one side may pass simultaneously through the mouth in an anterior to posterior sequence (Valentine & Glorioso, 1978). The significance of locomotion activities in the evolution of the pedal rubbing should be taken into account. Both actions, oral and/or pedal grooming of legs, appear to be performed in a similar manner to walking, and may be controlled by the same neural centre.

In Hymenoptera, many possible combinations of leg rubbing are present. The fore leg is used to clean the antennae, the other legs, the head and mesosoma, and in some cases, the metasoma as well. The middle-and hind legs are both used to clean the mesosoma, metasoma, wings and each other. The legs, in general, have some modifications which are specialized for particular cleaning functions. In many insects, the fore legs bear an antenna cleaning apparatus which, although mainly serving that function, may also be used to clean other appendages and/or body parts (Basibuyuk & Quicke, 1995 and refs. therein).

Behaviour 19. Fore leg nibbling. Usually the whole tarsus and apical portion of the tibia is drawn through the mouthparts; sometimes both fore legs are cleaned alternately this way (Fig. 4C). Cleaning fore legs by the mouthparts is the only grooming behaviour in the Hymenoptera that can be traced back to the common origin of the insects and myriapods (Jander, 1966). There is only one clearly established exception within the Hymenoptera: bees (Jander, 1976). This contrasts with Farish's (1972) observations which may be due to his failure to distinguish the grooming of fore leg by mouthparts from the grooming of mouthparts by the fore

leg as pointed by Jander (1976). The absence of this behaviour in bees appears likely to be a consequence of their mouthparts being variously modified for gathering nectar or other plant secretions, and this may be considered as an autapomorphy for bees. It would be interesting to know whether it has similarly been lost in the many parasitic wasps that have independently evolved mouth parts for nectar feeding. It has also not been recorded in the Tenthredinidae, Argidae, Siricidae, Proctotrupidae and Sapygidae, though evidence for these is not conclusive because of limited recording and sampling.

Behaviour 20. Fore leg–fore leg rubbing. The tarsus of one fore leg is passed along the tarsus of the other, or is passed through the antenna cleaner of other fore leg; the action may then be reciprocated (Fig. 2D). This behaviour is displayed by most taxa, but members of four symphytan families, (Cimbicidae, Diprionidae, Pergidae and Tenthredinidae) and members of helorid wasps appear not to perform this behaviour. Three chrysidoid wasp families (Chrysididae, Bethylidae and Dryinidae) and the Sapygidae and Sphecidae have not been observed to perform this behaviour. Absence of this behaviour may be due to secondary loss, and can be treated as a derived condition, though it should be noted that this could reflect insufficient recording time and intrafamilial variation.

Behaviour 21. Fore leg–fore leg–middle leg rubbing. The tarsi of fore both legs and a single middle leg are variously rubbed along each other and drawn under the tibial spurs (Wilson, 1979; Farish, 1972) (Fig. 4E). Behaviour 21 is characteristic of ants (Wilson, 1979) but, it has been observed in the Heloridae and in some Mutillidae (in rather rudimentary form). This behaviour was also reported for Proctotrupoidea and Chalcidoidea by Farish (1972).

Behaviour 22. Fore leg–fore leg–middle leg–middle leg rubbing. Both pairs of fore and middle legs are rubbed along each other (Wilson, 1962) (Fig. 5A). Behaviours 22 and 23 appear to be unique to ants (e.g. Farish, 1972; Wilson, 1962). However, it is interesting to note that a few female (wingless) mutillid and tiphiid wasps also occasionally perform behaviour 22, so it might be convergently evolved in apterous taxa.

Behaviour 23. Fore leg–fore leg–middle leg–hind leg rubbing. Usually arises from both fore legs and a middle leg rubbing, and then incorporating the hind leg from the same side as middle leg; it is unique for ants (Farish, 1972; Wilson, 1979) (Fig. 5B).

Behaviour 24. Ipsilateral fore leg–middle leg rubbing. Both fore leg and middle leg are rubbed along each other, or the fore leg may pass between the middle leg spur and basitarsus (Fig. 5C). This behaviour has not been observed in the Chrysididae, Dryinidae, Sapygidae and Sphecidae. Many male Mutillidae and possibly also the Xyelidae lack this behaviour whilst the presence of this behaviour in the Coleoptera and Diptera as well as in many Hymenoptera suggests that it may be the ancestral condition for the order. Its absence in the Xyelidae makes this less conclusive.

Behaviour 25. Ipsilateral fore leg passes through the femoral-tibial joint of the bent middle leg (Fig. 5D). Behaviour 25 has previously only been recorded for bees (Farish, 1972; Jander, 1976). It is also present in the Argidae, Cimbicidae and Pergidae, and in some cynipid and platygastroid wasps. From this patchy distribution and absence in basal sawflies, it appears to be a derived condition.

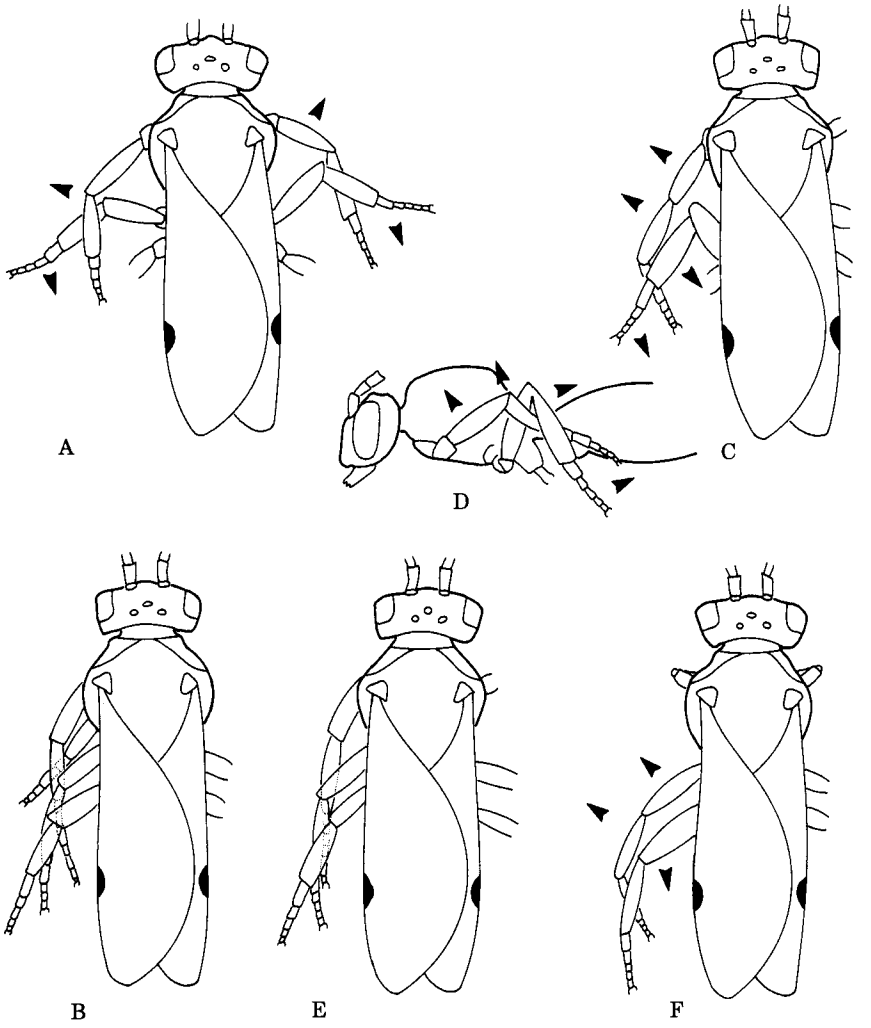


Figure 5. Leg rubbing. A, fore leg–fore leg–middle leg–middle leg rubbing (behaviour 22); B, fore leg–fore leg–middle leg–hind leg rubbing (behaviour 23); C, ipsilateral fore leg–middle leg rubbing (behaviour 24); D, ipsilateral fore leg cleaned by the femoral-tibial joint of the bent middle leg (behaviour 25); E, ipsilateral fore–middle–hind leg rubbing (behaviour 26); F, ipsilateral middle leg–hind leg rubbing (behaviour 27).

Behaviour 26. Ipsilateral fore leg–middle leg–hind leg rubbing. The tarsi of all three legs groom another along one side, though the hind leg usually remains motionless on the substrate while the other tarsi pass over it (Farish, 1972) (Fig. 5E). Behaviour 26 is only recorded in the Bethylidae and in some members of the Mutillidae. Farish (1972) also recorded this pattern in the Tenthredinoidea, Scolioidea and possibly in the Braconidae, and Wallis (1962) reported that while the hind leg was cleaned by middle leg, in ants the fore leg might also assist it. A similar grooming pattern has also been recorded in the Coleoptera (Valentine, 1973). Since no basal symphytan

is known to display this behaviour it is most parsimonious to assume that it evolved independently in the Hymenoptera.

Behaviour 27. Ipsilateral middle leg– hind leg rubbing. The tarsus of one leg is passed beneath the tibial spur and/or along the tarsus of the other leg, which then reciprocates the action (Fig. 5F). Behaviour 27 is not displayed in the symphytan families Xyelidae, Argidae, Tenthredinidae, Siricidae and Cephidae, the parasitic wasp family Heloridae, nor in the aculeate families Chrysididae, Dryinidae, Sapygidae, Tiphiidae, Pompilidae and Formicidae (e.g. Farish, 1972; Wilson, 1962, 1979); however, according to Wallis (1962) this behaviour was displayed by the ant, *Formica fusca* L. Absence of this behaviour in many symphytan families including Xyelidae suggests independent evolution within Hymenoptera.

Behaviour 28. Middle leg–middle leg–hind leg–hind leg rubbing simultaneously. This motion consist of the four legs mutually grooming one another (Fig. 6A). Behaviour 28 is performed by two symphytan families, the Argidae and Cephidae, as well as by members of the parasitic family Stephanidae, and the vespoid family Sapygidae, a few members of Mutillidae and a member of Platygastridae. Farish (1972) also reported that this pattern of grooming behaviour in the Vespoidea *s.l.*, Formicidae and Apidae. It is also known in the Coleoptera (Valentine, 1973), though the presence of this behaviour in several isolated taxa within the Hymenoptera suggests that it might be a derived condition.

Behaviour 29. Middle leg–hind leg–hind leg rubbing. The tarsus of each leg passes beneath the tibial spurs and/or along the tarsus of another leg (Fig. 6B). This behaviour was also found in the Diptera (Szebenyi, 1969). In the Hymenoptera, it occurs only in one symphytan family, the Argidae, though it is displayed by most members of most families of Apocrita. Farish (1972) did not record this behaviour in the Xiphydriidae, and it also appears to be absent in the Gasteruptiidae, Stephanidae, Pelecinidae, Diapriidae, Chalcidoidea and Bethylidae.

Behaviour 30. Hind leg–hind leg rubbing. The tarsus of one hind leg is passed beneath the tibial spurs and/or along the tarsus of the other hind leg, which then reciprocates the action (Fig. 6C). This behaviour pattern is displayed by almost all taxa. Apparent exceptions (cimbicids, diprionids, siricids, orussids and pompilids) are probably best regarded as being due to inadequate recording.

It is hardly possible to suggest an evolutionary progress line for each behavioural pattern, but in general terms it may be suggested that locomotion (stepping movements) has given rise to simple rubbing movements which involve two legs. Further, it is relatively easy to be convinced that the involvement of more than two appendages at a time is evolved from a simple rubbing motion of only two appendages involved.

Complex grooming (Behaviours 31–33)

These three complex behaviour patterns appear to be highly stereotyped and unique to Hymenoptera.

Behaviour 31. Fore leg–wing–metasoma grooming on dorsum. One fore leg at a time scrapes slightly raised wings and continues the movement to the basal segments of the metasoma (Fig. 6D). This behaviour is displayed by the Scelionidae and Platygastridae, and so provides some additional evidence to support monophyly of the superfamily Platygastroidea.

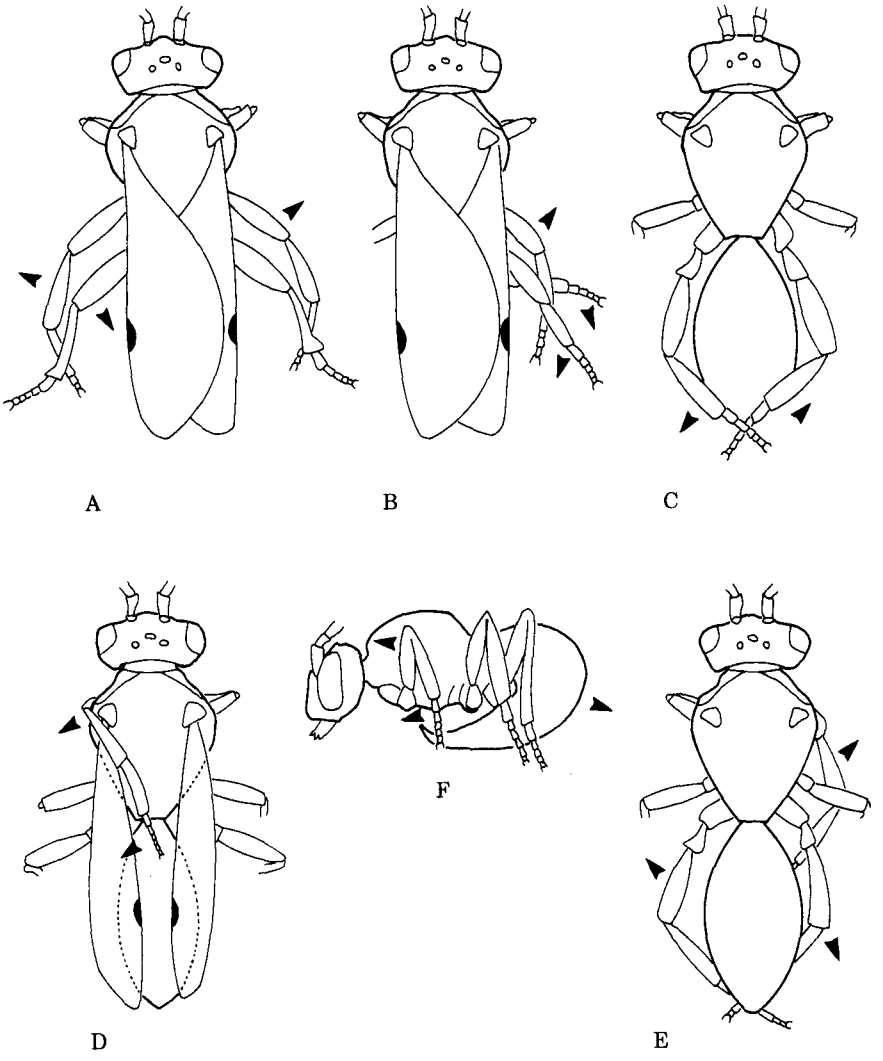


Figure 6. Leg rubbing (A–C) and complex grooming (D–F). A, both middle and hind legs rubbing simultaneously (behaviour 28); B, middle leg–hind leg–hind leg rubbing (behaviour 29); C, hind leg-hind leg rubbing (behaviour 30); D, fore leg–wing–metasoma grooming on dorsad (behaviour 31); E, fore leg–metasoma–hind leg grooming on ventrad (behaviour 32); F, metasomal tip grooming (behaviour 33).

Behaviour 32. Fore leg–metasoma–hind leg grooming on venter. During the performance of the metasoma grooming by hind legs, one fore leg may scrape the basal segments of metasoma ventrally, and may also touch the hind legs (Fig. 6E). This also appears as a prominent behaviour in the repertoire of the Scelionidae and Platygastridae and provides further evidence for their monophyly. A few apocritan Hymenoptera may occasionally perform this behaviour, though it is likely to be as a result of independent acquisition (Appendix 1).

Behaviour 33. Metasomal tip grooming. The metasoma bends forward between the legs and the head is bent down to reach the metasomal tip and then the tip is groomed by the mouthparts and/or foretarsi (Farish, 1972) (Fig. 6F). This was reported to be unique for ants within the Hymenoptera (Wallis, 1962; Wilson, 1962, Wilson, 1979; Farish, 1972), although it is also recorded here on one occasion for a male tiphiid wasp.

Phylogenetic implications

We entered the phylogeny proposed by Rasnitsyn (1988) as modified by Heraty, Woolley & Darling (1994), and Brothers & Carpenter (1993) (for Aculeata) into MacClade (Maddison & Maddison, 1992), and viewed each character using the trace option in order to examine the evolutionary patterns of grooming behaviours within the order. Only unambiguous changes under the parsimony criterion are presented in Figure 7. The overall consistency index (CI) was 0.22 and a minimum of 151 steps were required to fit the behavioural characters on the above phylogeny. The CI value is considerably lower than those found for some behavioural characters (de Queiroz & Wimberger, 1993). This may be partly due to incomplete recording of repertoires and limited sample size. However, it should be noted that the phylogeny employed may not be the most parsimonious one for the behavioural data and was constructed independently of behaviour. Nevertheless, several grooming behaviours appear to be potentially phylogenetically informative and these are discussed below. Also the CI is within the range of values obtained for several large morphological data sets for which the range of taxa investigated makes homoplasy more likely, and in which CIs have not been artificially inflated by the exclusion of more obviously homoplasious characters.

Monophyly of the Apocrita is supported by only one behaviour, the thorax scraping by fore leg (behaviour 7). The sister group relationship of the Orussidae and Apocrita is supported by the cleaning of the dorsal and ventral surface of the raised wing by the hind legs (behaviours 13 and 14). Grooming of pleura by fore leg (behaviour 10) supports the clade including Cephidae + (Siricoidea + (Orussidae + Apocrita)). Lack of behaviour 10 in the whole of the Aculeata may also be considered as a synapomorphy. Scraping the dorsal surface of raised wings by hind legs (behaviour 13) appears to have been lost along the branch giving rise to the tenthredinoid + cephid grade and subsequently to have been regained in the Orussidae + Apocrita (Fig. 7).

The cynipoid families and Chalcidoidea including Mymaridae were also united by several putative synapomorphies (Fig. 7). Further, monophyly of Platygastroidea was supported by behaviours 25, 31 and 32. Absence of double antenna scraping (behaviour 5) and thorax scraping by fore legs in the Pelecinidae and Proctotrupidae may suggest a close relationship between both families. Monophyly of the Ichneumonoidea was supported by a unique wing grooming behaviour (behaviour 16).

Absence of fore leg–fore leg rubbing supports monophyly of Chrysidoidea, though no putative apomorpies are found to support monophyly of either Vespoidea or Apoidea. Unsurprisingly, some of the highly specialized groups of aculeate Hymenoptera, such as ants and bees, have many unique grooming behaviours in their repertoire (Fig. 7).

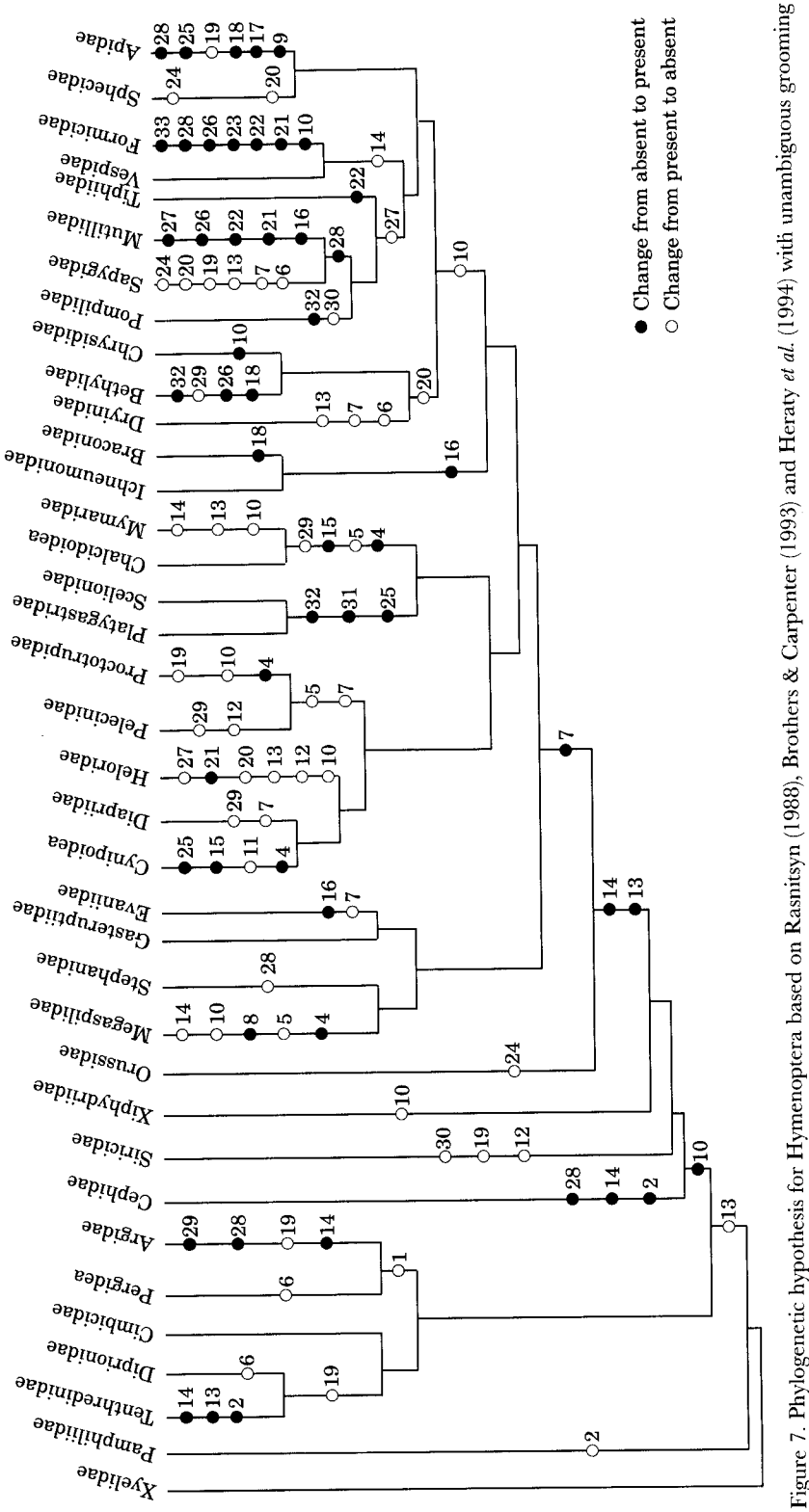


Figure 7. Phylogenetic hypothesis for Hymenoptera based on Rasnitsyn (1988), Brothers & Carpenter (1993) and Heraty *et al.* (1994) with unambiguous grooming behaviour character state changes superimposed.

Morphology of antenna cleaner and antennal grooming patterns

In recent years, several methods have become available to test for significant associations between two independent traits given a phylogenetic hypothesis (Ridley, 1983; Pagel & Harvey, 1989; Maddison, 1990). These methods require a phylogeny (assumed to be the best estimate of true phylogeny) and discrete characters (Harvey & Pagel, 1991). Ridley's method is designed to count the end-state of transitions for each branch in the tree and branches along which changes are not included. This method can be applied to determine whether an association between two characters has arisen through correlated evolution: that is, whether change in one is significantly correlated with that in another, perhaps because of dependency. It was not necessarily designed to detect the direction of evolutionary change within a phylogeny (Harvey & Pagel, 1991), although a test proposed by Maddison (1990) does. Harvey & Pagel (1991) described another method which can take into account the differing amounts of evolutionary change that might be expected to take place along branches of different lengths: long branches may indicate long time periods during which changes in many traits are likely to occur even if there is no connection between them.

Here, the primary question that has to be answered is whether there is any association between the morphology of the antenna cleaner and the patterns of antenna grooming (behaviours 1–5) within the Hymenoptera. Characters for the morphology of antenna cleaner are generated from Basibuyuk & Quicke (1995: 160), and recoded as binary characters (Appendix 3). Given a phylogeny, tallying the presence or absence of changes in behaviour and morphology for each branch throughout the tree gives a 2×2 contingency table from which χ^2 can be calculated (Siegel, 1956). The contingency table test could be biased because of uneven branch lengths, and if the results of this simple test suggest a strong association then one of the above more powerful tests may need to be considered.

Twenty-one morphological characters (Appendix 3) and five behavioural characters (behaviours 1–5) from antenna cleaner morphology and antennal grooming behaviour respectively, are included. Characters were traced using MacClade (Maddison & Maddison, 1992) on the best available phylogenetic tree (Rasnitsyn, 1988; Brothers & Carpenter, 1993; Heraty *et al.*, 1994), considering only those taxa for which antennal cleaning data is available. The number of unambiguous character state changes was calculated for each branch for behavioural and morphological characters separately. Trees showing the amount of change for behaviour and morphology are presented in Figure 8. The distribution of each morphological character was viewed in MacClade in order to allow for polymorphism occurring on an ancestral branch of a monophyletic group because it is assumed that such non-genetic polymorphism cannot be inherited from one taxon by another, and therefore must reflect multiple character state evolution within the terminal taxa.

The χ^2 test for two independent samples has been applied in order to determine the possible association between occurrence of character state changes in behaviour and morphology (Siegel, 1956). There are four possible combinations of changes in both sets of characters that can occur along a particular branch of the tree: (i) in behaviour, (ii) in morphology, (iii) in both, or (iv) in neither. Results are presented in Table 1. The probability of occurrence under null hypothesis for $\chi^2 \geq 0.064$ with $df = 1$ is $P > 0.40$. Thus there is no apparent correlation between the occurrence of morphological and behavioral changes. However, since this test only takes into account the presence or absence of changes rather than their magnitude, it could

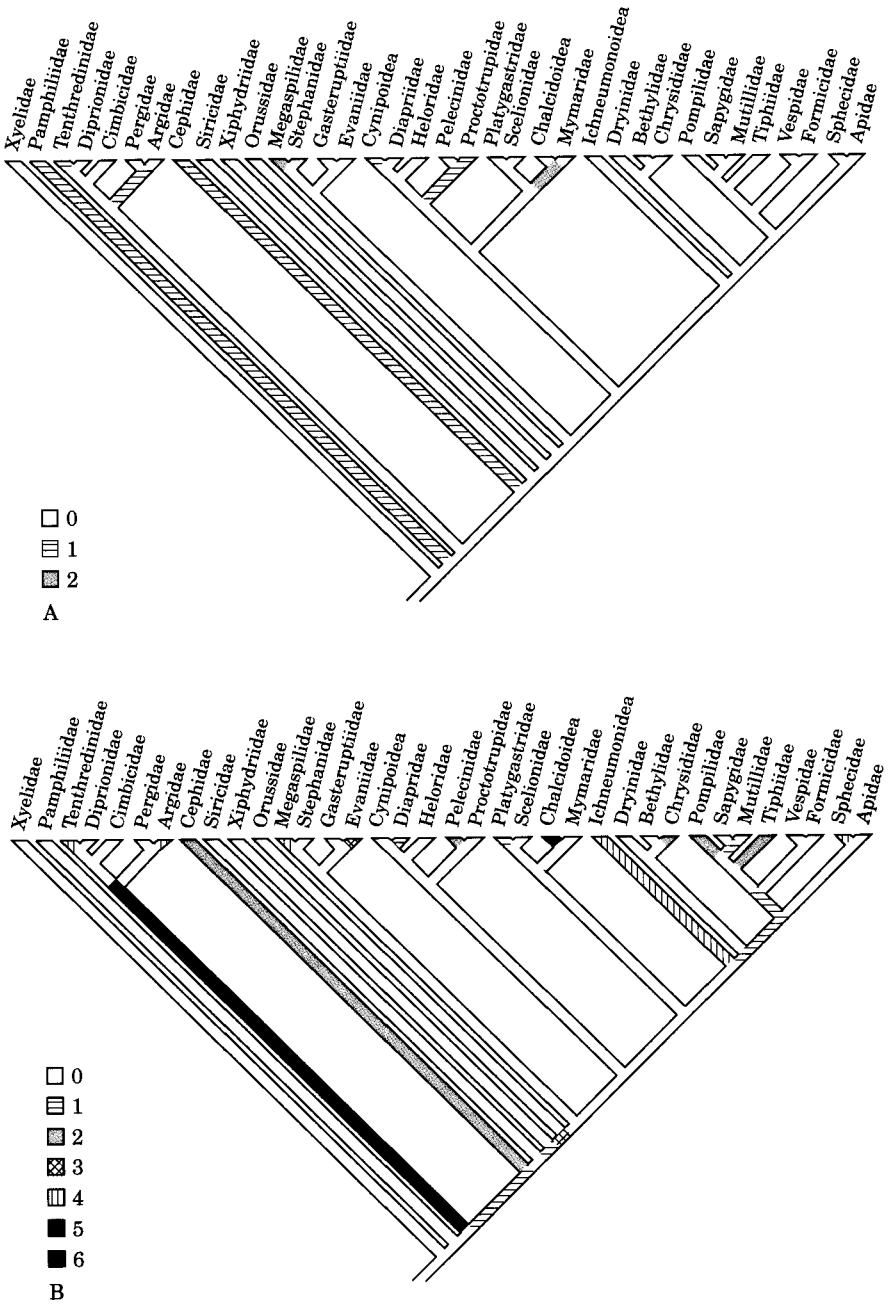


Figure 8. Total number of unambiguous changes in antenna grooming behaviour (A) and in antenna cleaner morphology (B).

fail to detect an association if there were a number of branches with considerable changes (i.e. changes in more than one trait) in morphology and behaviour. To test for this we calculated Spearman rank correlation coefficient (r_s) (Siegel, 1956).

Due to the considerable number of tied ranks for both morphology and behaviour,

TABLE 1. Pairwise comparison of presence and absence of character state changes in morphology and behaviour on branches of best available phylogenetic hypothesis

Characters	Behaviour−	Behaviour+	Total
Morphology−	42 (A)	6 (B)	48 (A+B)
Morphology+	19 (C)	3 (D)	22 (C+D)
Total	61 (A+C)	9 (B+D)	70 (N)

the tied observation correction was applied. Correction factor for each variable was calculated. Then, because there are considerable numbers of ties present, a suggested equation for this kind of case was used for computing r_s (Siegel, 1956). Calculation revealed that $r_s = 0$ ($P \gg 0.10$); thus there is no significant association between change in antennal grooming behaviour and antennal cleaner morphology, although the evidence is not conclusive partly because of absence of behavioural data for some major groups, insufficient sampling for some groups, and possible failure to record some of the antenna cleaning repertoire in some taxa. Therefore, why there are different antenna cleaning behaviours and what roles the various antenna cleaner structures play remain open questions. One possible explanation is that the efficiency of the cleaning of antenna may be related to cleaning frequency rather than cleaning behaviours. The utility of antennal grooming is to keep antennal sensilla clean for the environmental communication, and therefore more active species of insects appears to perform more frequent grooming (Jander, 1966). Another assumption concerning the utility of antennal grooming, however, is to press chemical signals onto the sensilla surface in order to facilitate their detection (Callahan, 1975). Recently, an antenna cleaner gland has been reported for an ant and its impact on grooming is not known (Schönitzer, Dott & Melzer, 1996). If grooming plays any role either in distribution or accumulation of chemical signals, the matter is more complicate and it will require further research. The question then would be whether different kinds of molecules require different manners of application.

CONCLUSIONS

Despite the fact that there have been numerous surveys of character systems within the Hymenoptera over the past decade or so (e.g. Gibson, 1985; Johnson, 1988; Whitfield *et al.*, 1989; Quicke *et al.*, 1992; Heraty *et al.*, 1994; Basibuyuk & Quicke, 1995, 1997; Vilhelmsen, 1996) current phylogenetic hypotheses for Hymenoptera, in particular those concerning deep relationships, are still very fragile. This is partly due to inclusion of morphological characters. Most of what we currently know comes from morphological studies; to date there have been only a few molecular data sets dealing with this issue (e.g. Dowton & Austin, 1994). There is considerable need for more characters to be investigated, and the behavioural ones described here appear capable of helping resolve some higher level relationships. However, no single character system is likely to yield an accurate or fully resolved phylogeny, especially in such a large and diverse group as the Hymenoptera. The best chance of determining evolutionary relationships will no doubt come from a

total evidence approach, and such future studies should include grooming behaviours as one of the data sets.

The phylogenetic implications of the grooming behaviour repertoires discussed here are rather tentative and a full account will only be possible following further observation. Nevertheless, possible paths in the evolution of grooming behaviours are revealed, and a framework is provided with the hope of encouraging further study.

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APPENDIX 1

List of material examined and their observed grooming repertoires. Explanation of symbols: (−) character not applicable; (F) female; (M) male; (?) missing data; (0) absent; (1) present

Taxa	Characters 1111111111222222222233333 12345678901234567890123456789012345
Xyeloidea	
Xyelidae:	
Xyela julii (Brébisson), 2F, 1M, U.K.	00001100001110100011000?000001000
Megalodontoidea	
Pamphiliidae:	
Cephalcia arvensis Panzer, 2M, 2F, Italy.	11101100001110000011000100100100 0
Tenthredinoidea	
Argidae:	
gen. sp., 1F, U.K.	00110100001101000001000110011100 0
Cimbicidae:	
gen. sp., 1F, U.K.	10000100001100000000000000010000 00
gen. sp., 1F, U.S.A.	10011100001100000010000110100000 0
Diprionidae:	
Diprion sp., 1M, U.K.	10100000001100000000000010010000 00
Pergidae:	
Phylacteophaga sp., 1M, 1F, Australia.	00110000001100000010000110100100 0
Tenthredinidae:	
Taxonus agrorum (Fallén), 1F, U.K.	11100100001111000000000100000100 0
Cephoidea	
Cephidae:	
Calameuta pallipes (Klug), 1F, U.K.	10001100011101000111000100010100 0
Trachelus troglodyta (Fabricius), 1F, France	00001000011001000000000000000010 00
Siricoidea	
Siricidae	
Tremex sp., 1F, U.S.A.	10001100001000000000010000000000 0
Tremex columba (L.), 1M, U.S.A.	10001100010000000101000100000000 0
Orussoidea	
Orussidae	
Orussus sp. 1M, U.S.A.	10001100011111000011000100100000 0
Evanioidea	
Evaniidae:	
Evania appendigaster (L.), 3F, U.S.A.	10001100011?110100110001001011000
Gasteruptiidae:	
Gasteruption assector (L.), 1F, U.K.	11001100001011000010000100100100 0
Gasteruption jaculator (L.), 1M, U.K.	10001110011011000011000100100100 0
Stephanoidea	
Stephanidae:	
gen. sp., 1F, U.S.A.	11001110011111000011000100110100 0
Cynipoidea	
Cynipidae:	
Diplolepis rosae (L.), 1F, U.K.	11010100010010100010000100001100 0
Neuroterus aprilinus (Giraud), 2F, U.K.	11010100000101000011000110100100 0
Synergus sp., 2F, U.K.	00000000000111000001000110101100 0
Saphonecrus connatus (Hartig), 1F, U.K.	11011100?000000001100010000011000
Charipidae:	
Alloxysta victrix (Westwood), 1F, U.K.	10000000000101000011000100101100 0
Alloxysta sp., 1?. U.K.	11000100000111100010000100001101 0
Ibaliidae:	
Ibalia sp., 2F, U.S.A.	11001110010111000011000100101100 0
Chalcidoidea	
Aphelinidae:	
Encarsia formosa Gahan, 2F, U.K.	00010110010100000011000100100100 0
Encyrtidae:	
Cheiloneurus elegans (Dalman), 3M, U.K.	00010000001000000011000100100100 0

continued

APPENDIX 1—*continued*

Taxa	Characters 1111111111222222222233333 12345678901234567890123456789123
Eulophidae:	
Diglyphus sp., 1F, U.K.	00000100011000000011000100100100
Pediobius sp., 1F, U.K.	00010110011100100010000100100100
Eupelmidae:	
Calosota aestivalis Curtis, 2F, U.K.	00010110001100100011000100100100
Macroneura vesicularis (Retzius), 1F, U.K.	00010110011-------110001001001000
Macroneura sp, 1F, UK	00010000001-------110001001001000
Eurytomidae:	
Eurytoma sp., 1M, U.K.	00010110011000000011000100100100
Sycophila biggutata (Swederus), 1F, U.K.	00010110011100100010000100100100
Mymaridae:	
Anaptes sp., 1M, U.K.	10010110001100100011000100100100
Mymar pulchellum Curtis, 1F, U.K.	00010100001000100001000100100100
gen.sp., 1?, U.K.	00010110001100100011000100100100
Pteromalidae:	
Callitula sp., 1F, U.K.	00010110011-------110001001001000
Gastracanthus pulcherrimus, Westwood, 1F, U.K.	00000010001001100011000100100100
Habritys bervicornis (Ratzeburg), 1F, U.K.	00010000000110100010000000000100
Torymidae:	
Torymus sp., 1F, U.K.	00000110011100100011000100100100
Torymus sp., 3F, U.K.	00000110011000100011000100100100
Platygastroidea	
Platygastridae:	
gen. sp.	00000000001011000000001000011110
gen. sp.	1?011100111000000110001101111100
gen. sp.	11000010001011000010000100000000
gen. sp.	11001010001000000010000100010000
Leptacis sp., 2?, U.K.	110?11100110100000110001101011010
Platygster munita Walker, 1F, U.K.	00000000001000000000000100001000
Scelionidae:	
Telenomus, 1?, UK.	11001100011000000010000110101101O
Telenomus busseola Gahan, 3F, 1M, Italy	11001110011111000011000100001111O
Trimorus sp., 1F, U.K.	11001010011?10000100001101011110
Trissolcus sp., 3F, U.K.	11001110011111000011010100101111O
Proctotrupoidea	
Diapriidae:	
Aclista sp., 1F, U.K.	10001100000000000010000000000000
Belyta sp., 1F, 1M, U.K.	01001100011000000010000100100100O
Spilomicrus sp., 1F, U.K.	100?00000000000000110001001001000
Coptera occidentalis Muesebeck, 3F/M, N.America	11001100001111000011000100100100O
Heloridae:	
Helorus sp., 1F, U.K.	?????11000100100001010010000110O0
Pelecinidae:	
Pelecinus polyturator (Drury), 1F, Australia.	11000100011011000011000100100100O
Proctotrupidea:	
Codrus niger Panzer, 1F, U.K.	10010100001111100001000100101100O
Ceraphronoidea	
Megaspilidae:	
Dendrocerus carpenteri (Curtis), 1F, U.K.	1?01011100101000001100010010110O0
Lagynodes sp., 1F, U.K.	10000100000-------100001000000000
gen. sp., 1F, U.K.	00010100000010000010000000000100O
Ichneumonoidea	
Ichneumonidae:	
Ephialtes manifestator (L.), 1F, U.K.	11000100000000000010000000000000
Odontocolon sp., 1F, U.K.	1010?10001111101O?110000000001000
Ophion minutus Kriechbaumer, 1F, France	11000010001010000001000000101100O
Orthopelma sp., 2F, U.K.	11001110011011000011000100001000O
gen.sp., 1F, U.K.	11011100011011000011000100001100O
gen. sp. (Diplazontinae), 1F, U.K.	01001000000000000010000000001000

continued

APPENDIX 1—*continued*

Taxa	Characters 1111111111222222222233333 1234567890123456789012345678901234
Braconidae:	
Aphidius sp., 1F, U.K.	11000000001001000011000000001000
Atanycolus sp., 1F, France	11000110011000000111000000101101 0
Blacus sp., 1F, U.K.	00000000000000000010000000001000 0
Chelonus sp., 2M, U.K.	11001100001111010011000100001100 0
Diaeretiella rapae (M'Intosh), 1F, U.K.	11000100000000000011000100101100 0
Dorctes sp., 3F, U.K.	11000100011111000011000100001100 0
Hecabolus sulcatus Curtis, 1F, U.K.	10000000000000000010000000001000 0
Histeromerus mystacinus Wesmael, 3F, U.K.	11001100001000000010000110?11000
Homolobus sp., 1F, U.K.	11000110000000000011000100000100 0
Meteorus sp., 1F, U.K.	10000100001000000011000100001100 0
Polemochartus liparae (Giraud), 1F, U.K.	11000100011101000011000100001100 0
gen. sp. (Microgasterinae), 1F, U.K.	11000110000000000010000000001000 0
Chrysidoidea	
Bethylidae:	
Goniozus claripennis (Förster), 1F, U.K.	10001110001011000010000101100101 0
Epyris sp., 1F, France	10001000001000000110000000000100 0
Chrysididae:	
Chrysis sp., 1F, U.K.	00001110010000000010000000001000 0
Chrysogona sp., 1F, France	10001010001110000010000000001000 0
Trichrysis cyanea (L.) 1F, U.K.	10001110011011000010000000001100 0
Dryinidae:	
Chelogynus sp., 2M, U.K.	10001000001001000010000000001100 0
Sapygidae	
gen. sp. 1F, U.K.	00000000001101000000000000011100 0
Vespoidea	
Mutillidae:	
Pseudomethoca sanbornii acetis (Fox), 1F, U.S.A.	11001110011-------100001000011000
Timulla floridensis (Blake), 1F, U.S.A.	10001110011-------000001011111010
gen. sp., 1F, U.S.A.	10001100011-------010001000011000
gen. sp., 1F, U.S.A.	10000110011-------100001011000000
gen. sp., 1F, U.S.A.	10001000000-------000001000000000
gen. sp., 1F, U.S.A.	10001110011-------101101101010000
gen. sp., 1F, U.S.A.	10000000000-------100000101000000
gen. sp., 1F, U.S.A.	10000110011-------100001001011000
Dasymutilla lepeletierri (Fox), M, U.S.A.	10000100001000000010000100001000 0
Tricholabiodes thisbe (Péringuey), M,S. Africa[1]	10001110011010010010000100011100 0
gen. sp., 1M, U.S.A.	00001000001000000001000000000100 0
Odontophotopsis sp., 1M, U.S.A.	10001000001000000000000000000000 0
Tiphiidae:	
Brachycistis sp., 1M, U.S.A.	10001110011000000010000100101100 1
Colocistis sp., 1M, U.S.A.	11000110010000000011000101000000 0
Myrmosa atra atra Panzer, 1F, U.K.	10001110011-------010000100000000
Myrmosa sp., 1F, U.K.	10000110011-------100101000001000
Pompilidae:	
Anoplius sp., 1F, U.K.	00001100011011000010000100001000 0
Ceropales sp., 1F, U.K.	10000110?10111000110000000010010
Apoidea	
Sphecidae:	
Diodontus Curtis., 1F, U.K.	10001100001001000010000000101100 0
gen.sp., 1F, U.K.	00000100001000000010000000001100 0

[1] Bayliss & Brothers (1996).

APPENDIX 2

Grooming behaviour repertoire of some selected taxa of the Hymenoptera. Explanation of symbols: (−) character not applicable; (?) missing data; (0) absent; (1) present.

Taxa	Characters 1111111111222222222233333 12345678901234567890123456789123
Xyelidae	000011000011107000110007000001000
Pamphiliidae	111011000011100000110001001001000
Argidae	001101000011010000010001100111000
Cimbicidae	100111000011000000100001101000000
Diprionidae	101000000011000000000001001000000
Pergidae	001100000011000000100001101001000
Tenthredinidae	111001000011110000000001000001000
Cephidae	110011000111010001110001000101000
Siricidae	100011000110000001010001000000000
Xiphydriidae[1]	107?110000110000001100017?01011000
Orussidae	100011000111110000110001001000000
Gasteruptiidae	110011100110110000110001001001000
Evaniidae	100011000117?1101001100010010011000
Stephanidae	110011100111110000110001001101000
Cynipoidea	110111100101111000110001101101107?0
Chalcidoidea	000101100111111000110001001001000
Mymaridae	?001011000110010001100010010010000
Platygastridae	110?111001111100001100011017?11110
Scelionidae	110011100111110000110?011?1011110
Diapriidae	110011000?111100001100010010010000
Heloridae	?????110001001000010100100001100 0
Pelecinidae	110001000110110000110001001001000
Proctotrupidae	100101000011117?000010001001011000
Megaspilidae	100101110010100000110001001011000
Ichneumonidae	110011?001111101001100010010110000
Braconidae	110011100?11?101011100010010110000
Bethylidae	100011110010110001100001011001010
Chrysididae	100011110110110000100000000011000
Dryinidae	100010010010010000100000000011000
Sapygidae	?????00?001?010000000000000111000
Mutillidae	100011100110110100111101011111000
Tiphiidae	10001111001-------110101000011000
Vespidae[1]	100011100010100000110001001011000
Formicidae[2]	100011100110100000111111011111001
Pompilidae	100011100110117?000110001000010010
Sphecidae	100011110011110000100000001011000
Apidae[3]	100011111?111100110100011011110000

[1] Farish (1972)
[2] Wallis (1962); Wilson (1962, 1979)
[3] Farish (1972); Jander (1976); Jander & Jander (1978)

APPENDIX 3

Data matrix re-coded as binary characters from Basibuyuk and Quicke (1995: 160). Character number is given in the first two rows. p = polymorphic. ? = missing data or character not applicable. Defination of characters: 1 – Posterior apical spur: 0 = present; 1 = absent. 2 – Posterior apical spur (if present): 0 = well-developed; 1 = reduced. 3 – Shape of anterior spur: 0 = modified; 1 = unmodified. 4 – Distal part of trunk of anterior spur (calcar): 0 = divided; 1 = undivided. 5 – Distal part of trunk of anterior spur (calcar): 0 = bifurcate; 1 = trifurcate. 6 – Anterior spur (calcar); 0 = curved; 1 = straight. 7 – Calcar. 0 = with velum; 1 = without velum. 8 – Velum (if present): 0 = smooth; 1 = with a spinose or ribbed surface. 9 – Rim of velum: 0 = smooth; 1 = modified (not smooth). 10 – Modified rim: 0 = with serrated rim; 1 = with a fine comb of socketed setae along edge. 11 – Calcar: 0 = without apex; 1 = with apex. 12 – Basitarsal notch: 0 = absent; 1 = present. 13 – Basitarsal notch: 0 = weak 1 = well-developed. 14 – A structure for purpose to clean antenna developed on basitarsal posteriorly: 0 = absent; 1 = present. 15 – A protocomb of erect but well spaced setae on basitarsi posteriorly: 0 = absent; 1 = present. 16 – A comb of closely set, fine setae on basitarsi posteriorly: 0 = absent; 1 = present. 17 – A row of plate-like structures on basitarsi posteriorly: 0 = absent; 1 = present. 18 – Basitarsus anteriorly: 0 = with setae; 1 = without setae. 19 – Basitarsal setae; 0 = somewhat modified; 1 = ordinary. 20 – Shape of modified basitarsal setae: 0 = paddle-shaped; 1 = blunt or filiform. 21 – Row of modified setae: 0 = one; 1 = more than one.

Taxa	Characters 111111111122 12345678901 2345678901
Xyelidae	000000000?00?00000000
Pamphiliidae	000000000?00?00000000
Argidae	0011?11???00?00000???
Cimbicidae	0011?11???00?00000011
Diprionidae	0011?11???00?00000011
Pergidae	0011?11???00?00000011
Tenthredinidae	000000000?00?000001??
Cephidae	1?0000000?00?11000000
Siricidae	1?0000000?00?00000000
Xiphydriidae	P10000000?00?00000001
Orussidae	P10000000?01010100001
Evaniidae	1?0000010?01010011???
Gasteruptiidae	1?0000000?01010100011
Stephanidae	1?0000000?01010100001
Cynipoidea	1?0000000?01010101???
Chalcidoidea	1?p00pp00?0p0p01000p1
Mymaridae	1?0000000?01010101???
Platygastridae	1?0010000?01010101???
Scelionidae	1?0000000?01010101???
Diapridae	1?0000000?01010100011
Heloridae	1?0000000?01010101???
Pelecinidae	1?01?0000?01110101???
Proctotrupidae	1?0000000?01010101???
Megaspilidae	000000000?01010101???
Ichneumonoidea	?0p00p0p1p101010p01p1
Bethylidae	1?01?0000?01110100001
Chrysididae	1?01?0001011110100001
Dryinidae	1?0000000?01110101???
Sapygidae	1?0000000?01110101001
Mutillidae	1?0p0p000?p111010p001
Vespidae	1?01?0001111110100001
Tiphiidae	1?0p0p00101111010p0p1
Formicidae	1?01?0001111110100001
Pompilidae	1?01?1001111110100011
Sphecidae	1?01?000p1p111010p011
Apidae	1?01?0000?11110100001