

Gall Midges (Diptera: Cecidomyiidae) are Vectors for their Fungal Symbionts

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Abstract

The female adults of three groups of Cecidomyiidae have evolved specialized structures to transport conidia of *Macrophoma* or related taxa. At least for some of these cecid species, the gall midges depend on the inoculated fungi as food for larvae developing within galls.

Conidia are carried in conspicuous, elongate pockets on abdominal segment 9 in females of most Alycaulina. In the Lasiapterina, conidia are entrapped among well developed setae on segment 8 and the cercus. In the Asphondyliidi, conidia are carried in a membranous sac dorsal to abdominal sternite 7 and opening along the posterior margin of that sternite.

These different structural adaptations to transport conidia and the phylogenetic relationships of the three cecid taxa indicate that an interdependence of each group with *Macrophoma*-like fungi may have evolved independently in these three groups of gall midges.

Keywords: Cecid, vector, symbiosis, *Asteromyia*, *Asphondylia*, *Lasioptera*, *Macrophoma*.

1. Introduction

Only a few instances of a mutualistic relationship have been discovered between arthropods and fungi in which the arthropod serves as vector of the fungus (Batra and Batra, 1979; Graham, 1967; Kukor and Martin, 1983; Lindquist, 1985; Morgan, 1968; Weber, 1979). In all of these cases the fungus is transported by adult females of the vector and is subsequently utilized as food for the immatures and sometimes also the adult.

An association between the larvae of certain cecidomyiid midges and fungi has been recorded by previous workers. Docters Van Leeuwen (1929, 1939) was first to provide details of an apparently mutualistic relationship between an East Indies midge, *Asphondylia bursaria* Felt, and an unidentified fungus growing in a gall on *Symplocos fasciculata* Zollinger. Fungal conidia were introduced into the host plant at the time of oviposition. Newly hatched larvae suspended development until fungal mycelium was well established in the gall and then proceeded to feed exclusively on the fungus. Associations between fungi and midges have been noted for species of the following cecidomyiid genera: *Asphondylia* Loew (Goidanich, 1941; Neger, 1910; Puzanova-Malysheva, 1935), *Lasioptera* Meigen (Kaiser, 1978; Meyer, 1952), *Calamomyia* Gagné (Gagné, 1981), *Neolasioptera* Felt (Cosens, 1912), and *Asteromyia* Felt (Batra, 1964; Batra and Lichtwardt, 1963; Gagné, 1968; Trelease, 1884; Weis et al., 1983). Although Docters Van Leeuwen (1929, 1939) and Puzanova-Malysheva (1935) have previously noted that the *Asphondylia* species they studied introduced fungal conidia into the host plant, no author has elucidated exactly how the fungus is transported.

2. Materials and Methods

Almost all specimens examined for this study were collected with Malaise traps into 70% ethanol. Others were present as pinned or slide mounted specimens in the collections noted below.

Specimens on microscope slides were first treated successively with KOH, acetic acid, cedarwood oil and then mounted in Canada Balsam. Specimens examined with the SEM were critical point dried from 98% ethanol, sputter coated with gold and examined with a Cambridge Stereoscope MK II.

In our survey of the distribution of spore carrying structures amongst cecidomyiid midges, we examined material from the Canadian National Collection, Ottawa, Canada, and from the United States National Museum, Washington, D.C., USA.

3. Results and Discussion

The female adults of three groups of Cecidomyiidae possess specialized conidia carrying structures, each different from one another, which ensure inoculation of a specific fungus onto the host plant during oviposition.

The first type of conidia carrying structure is exhibited by some members of the subtribe Alycaulina, a group that is restricted to the New World, and includes the genus *Asteromyia* Felt. Species of *Asteromyia* are gall midges which form blister galls on the leaves and stems of Astereae. It has long been known that a fungal growth is associated with the larval galls and that neither insect nor fungus has been observed independently of the other (Batra, 1964; Batra and Lichtwardt, 1963; Osten Sacken, 1862; Trelease, 1884). Larvae are

situated within the fungal stroma and probably feed on the fungus. Most species of *Asteromyia* have several generations per year. Larvae overwinter in the gall, pupate in the spring, and the adults emerge shortly thereafter (Gagné, 1968; Weis et al., 1983).

During the summers of 1983 and 1984 the senior author collected local free flying cecidomyiid midges with a Malaise trap. Females of *Asteromyia tumifera* (Beutenmüller), *A. modesta* (Felt) and *A. carbonifera* (Osten Sacken), collected in May, June and July all carried conidia in a pair of dorsolateral, elongate pockets on segment 9 (Fig. 1a).

Females have a telescopic type of ovipositor (Fig. 1a). In the retracted state, segments 8 and 9 are contained by segment 7, with the terminal cercus externally visible at the apex of segment 9. The conidia pockets are elongate and supported internally by thickened cuticle. The pockets and their contained conidia are easily seen in cleared specimens when these are mounted dorsoventrally.

Conidia carried by *Asteromyia* were more or less ellipsoidal, smooth-walled, hyaline to brownish and 0 or 1-septate (rarely 2-septate). The conidial base was truncate and relatively thin-walled, indicative of holoblastic ontogeny. These observations were characteristic of the genus *Macrophoma* (Sacc.) Berl. and Vogl. Specifically, the fungus carried by *Asteromyia* may be *Macrophoma gallicola* Sacc. The type collection on leaf blister galls on *Solidago mollis* from North Dakota was examined. Conidia were pale brown and mostly aseptate, and although apparently less mature, were otherwise the same as the conidia carried by *Asteromyia*.

We reared females of *A. carbonifera* and *A. euthamiae* Gagné directly from *Solidago* galls in July and August 1984 and, although they had conidia pockets, none had conidia present in these. Our observations on leaf galls on Astereae indicate that the associated fungus does not fruit on the galls or other parts of infected plants at the times when adult flies are emerging. Clearly, our field collected females must have picked up spores, possibly from ground litter or even from some other host plant species, after they emerged from their gall. This may explain why previous workers have had little success in attempting to get reared females to induce galls in proffered plants (Gagné, 1968; Weis et al., 1983).

We examined other members of Alycaulina and found similar conidia pockets containing conidia of *Macrophoma* in members of the following genera: *Neolasioptera*, *Calamomyia* (Fig. 1b-d) and *Chilophaga*. Occasionally, in addition to the numerous conidia of *Macrophoma*, one or a few conidia or urediniospores of other fungal genera were observed in the conidia pockets.

Empty but well developed conidia pockets were found in *Astictoneura* Gagné and *Edestochilus* Gagné. Although nearly all field-collected specimens were carrying conidia and nearly all reared specimens were not, there were some exceptions. Females of *Neolasioptera cornicola* (Beutenmüller) reared from *Cornus stolonifera* Michx. twig galls, and *N. willistoni* (Cockerell) reared from *Atriplex canescens* (Pursh) Nutt. twig galls were carrying conidia of *Macrophoma* which they undoubtedly picked up directly from their gall upon emergence. Associated unnamed fungi have been previously reported from the galls of species of *Neolasioptera* (Cosens, 1912) and *Calamomyia* (Gagné, 1981). Conidia pockets were lacking in members of *Protaplonyx* Felt and were poorly developed (or absent?) in *Meunieriella* Rubsaamen.

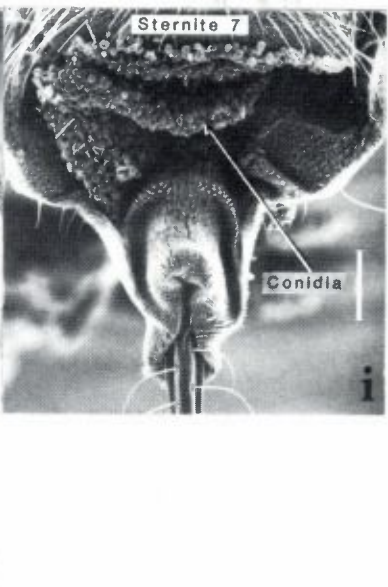
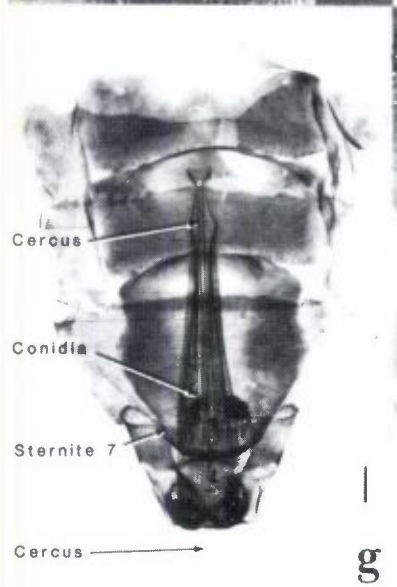
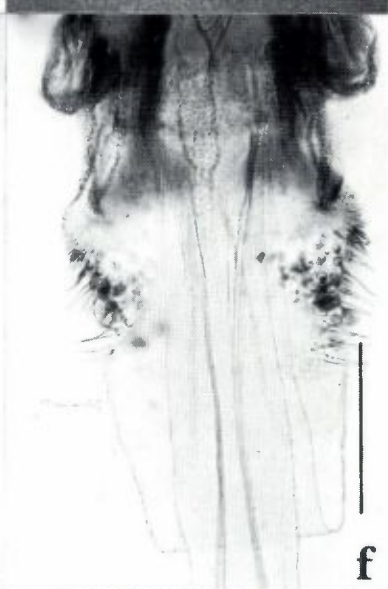
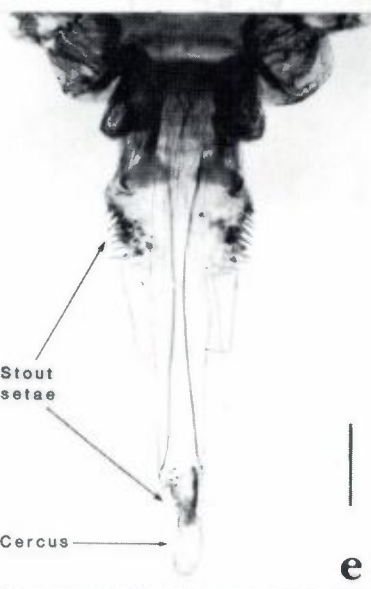
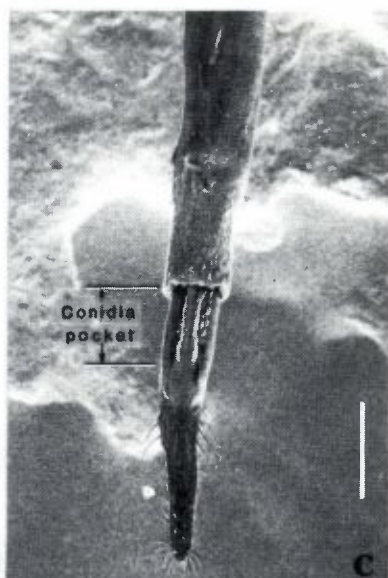
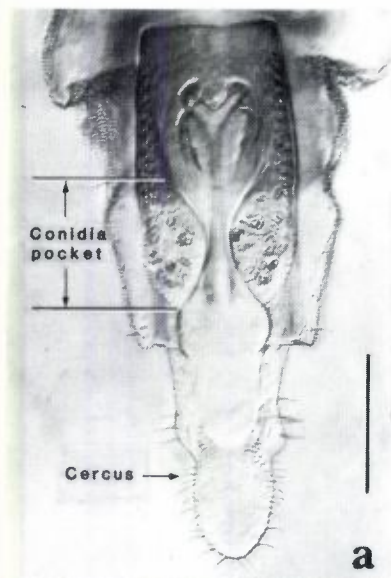
The second type of conidia transferring mechanism is exhibited by at least some members of the subtribe Lasiopterina. Previous workers have noted an associated fungus in galls of some of these species (Kaiser, 1978; Meyer, 1952). We found females of *Lasioptera* and *Ozrhincus* Rondani carrying *Macrophoma* conidia. Like members of Alycaulina, *Lasioptera* and *Ozrhincus* females have a similar protrusible ovipositor (Fig. 1e,f). Instead of the Alycaulina type pockets, they have two dorsolateral groups of strongly developed setae situated at about 1/3 the length of segment 8 and similar setae on the cercus. When the ovipositor is retracted, each set of setae on segment 8 folds in on itself to form a lateral pocket in which conidia are entrapped (Fig. 1f) as well as being apposed to the well developed setae on the cercus which also entrap many conidia.

The third kind of conidia carrying structure is present in some members of the supertribe Asphondyliidi. Females of *Asphondylia* and *Schizomyia* Kieffer have long, needle-like ovipositors (modified cerci) which, when retracted, are ensheathed within the abdomen. Specimens of *Polystepha* Kieffer also exhibit elongate ovipositors but these are not as well developed as in the first two genera. As in all members of the supertribe, sternite 7 is well developed and longer than sternite 6 (Fig. 1g).

We examined females of *Asphondylia*, *Schizomyia*, and *Polystepha* from localities as distant as Argentina, Israel and Australia, and discovered that

Figure 1. Conidia carrying structures on ovipositors of Cecidomyiidae

(a) *Asteromyia tumifica* in dorsal view, from Ancaster, Ontario; (b) *Calamomyia* in dorsal view from Metcalfe, Ontario, (c-d) *Calamomyia* in dorsal view from Kemptville, Ontario; (e-f) *Lasioptera* in dorsal view from Deganya, Israel; (g-h) *Asphondylia* in ventral view from Agua Caliente, Jujuy, Argentina; (i) *Schizomyia* in ventral view from Cabin John, Maryland, USA. Scale on photos = 0.10 mm.



nearly every field-collected female carried conidia in a membranous sac positioned dorsal to the posterior portion of abdominal sternite 7 (Fig. 1g-i). The sac shape varied from species to species but always extended laterally to, or nearly to, the margins of sternite 7 and opened along the posterior margin of the sternite. This unusually well developed sternite is probably used as a "shovel" to scoop up conidia. In all specimens examined, the majority of the conidia were referable to *Macrophoma* or to related taxa, but conidia of *Cladosporium*, (?) *Aplosporella*, *Bispora*, *Taeniolella* and *Alternaria* were also sometimes present. Conidia of the latter genera, all of which are common leaf and litter fungi, were probably collected incidentally by the relatively indiscriminate "scooping" method of collection likely employed by female Asphondyliidi. Setae and miscellaneous detritus were also present in the sacs of some specimens. Several specimens contained a nearly intact fungal stroma bearing conidiogenous cells and attached immature conidia of a *Macrophoma*-like fungus.

Although there are relatively few records of fungi present in the galls of cecidomyiid midges, our descriptions of conidia carrying structures suggest that all members of the genera described above will have associated fungi. Some of these cecid genera are composed of many species (e.g. *Neolasioptera*, with 110 spp., and *Asphondylia* with over 200) and larvae of these attack a wide array of hosts, from grasses to composites. It seems clear that a fungus-cecidomyiid relationship is widespread and involves hundreds of midge species. In addition, cecid taxonomy is still in its earliest stages and it is highly probable that many more species will be discovered.

We believe that the conidia carrying mechanism of the Lasiopterini and of the Asphondyliidi are independently derived for two reasons. First, the structure of the conidia pockets and ovipositor are morphologically very different in the two groups and are not apparently derived from homologous structures. We do not see how they could form an evolutionary morpholine. Secondly, the Lasiopterini are more closely related to the Ledomyiini and Oligotrophini than they are to the Asphondyliidi. Both Ledomyiini and Oligotrophini females appear to lack conidia carrying structures (Fig. 2).

The Alycaulina is the sister group of Lasiopterina; together they form the tribe Lasiopterini. Although both groups carry their conidia in a quite different manner, it is conceivable that each developed a specialization from a more simple method of carrying conidia. We note however that some Alycaulina lack conidia pockets and if these were shown to be the primitive

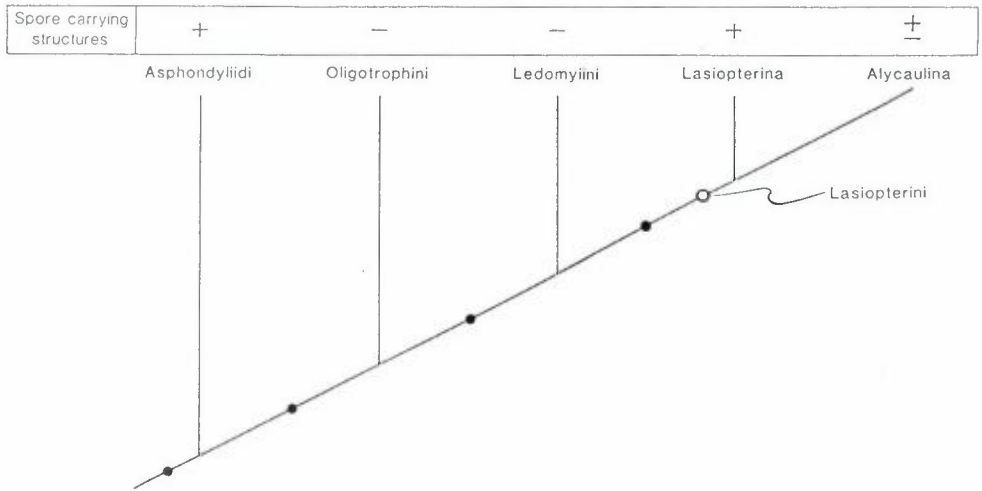


Figure 2. Phylogenetic relationships between some groups of Cecidomyiidae.

sister group to other Alycaulina, this would indicate that the two subtribes evolved their conidia pockets independently.

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