The tympanal hearing organ of a fly: phylogenetic analysis of its morphological origins

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Abstract. A key adaptation for any parasitoid insect is the sensory modality that it uses to locate its host insect. All members of the speciose family Tachinidae (Diptera) are parasitoids, but only flies of the tribe Ormiini use acoustic cues to find their hosts. Ormiine flies are parasitoids of various genera of crickets and katydids. Gravid females of one ormiine species, Ormia ochracea, hear the reproductive calling song of male field crickets and home in on those calls to locate their hosts. While many flies possess various kinds of “ears” to detect airborne sounds, only ormiine flies have been reported to possess true tympanal hearing organs. Such organs are well-known to occur in their cricket and katydid hosts. The ormiine ear is an evolutionary innovation within Diptera. Our objective was to trace the phylogenetic origins of the tympanal hearing organ among higher flies. Since the ormiine hearing organ is a complex organ within the prothorax, we examined possible precursor structures in the prothoraces of selected Diptera. We have uncovered a suite of characters that define the ormiine ear. These characters in the prothorax include a pair of prosternal tympanal membranes, a pair of chordotonal sensory organs, and modifications of the tracheal system. We have been able to identify and trace the presumptive homologs of these ormiine characters through selected species of related Diptera, using the method of outgroup comparison.

Key words: Chordotonal organ – Tracheal system morphology – Parasitoid Tachinidae – Ormia ochracea – Myiopharus doryphorae – Eurosta solidaginis – Neobellieria bullata (Insecta)

Introduction

In host-parasitoid relationships the ability of the parasitoid to find its host is crucial for its survival. Insect parasitoids typically rely on visual and chemical cues to localize their hosts. A few parasitoids, however, use auditory signals emanating from their hosts. One such parasitoid is the fly Ormia ochracea (Diptera, Tachinidae, Ormini, Robert et al. 1992, 1994a). In this species, the female fly seeks out its orthopteran host, a male cricket such as Gryllus rubens or G. integer (Orthoptera, Gryllidae), as the cricket calls to attract female conspecifics (Cade 1975; Walker 1986). The female parasitoid fly homes in on the male cricket’s calling song. The fly then larviposits on or near its host. These first instar larvae burrow into the cricket’s body and develop and grow within their host, consuming only selected tissues (Adamo et al. 1995). After 7–10 days, the third instar larvae burrow out of the host, pupate, and eventually emerge as tympanate adult flies. The host dies shortly after the fly larvae leave the cricket’s body (Adamo et al. 1995).

The tympanal hearing organ in O. ochracea can be divided into several distinct and essential morphological components. The essential structures of the fly’s or any insect’s tympanal organ include an external flexible tympanal membrane and related cuticular structures, an air sac in apposition to the tympanal membrane, and a sensory organ coupled to the tympanal membrane (Robert et al. 1994a). In insects, auditory sensory organs fall into the general category of chordotonal organs (Dethier 1963). These organs are internal sensory organs comprised of one or more scolopidia, the individual sensory units of the chordotonal organ (Howse 1988; Moulins 1976; McIver 1985). Because of their characteristic morphology, they are readily identifiable in histological preparations. Chordotonal organs function as stretch or vibration receptors and can respond to movements of nanometer amplitudes (French 1988).

Although a few species closely related to O. ochracea, namely, ormiine tachinids, are also tympanate (Cade 1975; Walker 1986), the majority of tachinid spe-
cies are atympanate. In *O. ochracea* the tympanal hearing organ is located in the ventral prothorax just below the cervix. However, little is known about the morphology of this region in other species of Diptera. The adult tracheal system is described in relative completeness only in *Drosophila melanogaster*, a member of the Acalypratae (Miller 1950; Whitten 1980), but not for any member of its speciose sister group the Calyptratae, which includes such families as Tachinidae, Sarcophagidae, Calliphoridae, and Muscidae. Similarly, little is known about the internal sensory elements of the ventral prothorax. A chordotonal organ has been observed in the ventral prothorax of *Drosophila melanogaster* (Hertwick 1931; Power 1948; Miller 1950) and *Calliphora erythrocephala* (Vater 1961; Strausfeld et al. 1987), but the relationship of that sensory organ to the auditory organ of *O. ochracea* is unclear.

The tympanal organ in ormiine flies is a key evolutionary innovation that allowed for a radiation of parasitoid flies into a niche that includes acoustically active hosts. These ormiine flies offer a unique opportunity for phylogenetic analysis. First, comparisons of the novel trait in the endowed taxon provide information regarding features of the tympanal organ that may be modified in different ormiine species. Second, we can investigate the phylogenetic precursors of this organ. This study focuses on the evolution of the tympanal hearing organ in tachinid flies. Because of the potential homologies between the tympanal hearing organ of *O. ochracea* and the thoracic structures in related calyptrate and acalyprate flies, we compared the thoracic anatomy of three atympanate and one tympanate species of Diptera selected on the basis of phylogenetic relatedness. We focused on the features necessary for a functional tympanal hearing organ: a prosternal tympanal membrane, an acoustic chamber and related tracheal structures, and an associated chordotonal sensory organ. We find that some features are common to all four species studied, while other features are found only in some species. The evolutionary implications of these results are discussed. Some of the results described here have been presented in abstract form (Edgecomb et al. 1994).

### Materials and methods

#### Animals

Four species of adult flies were studied: three representatives of the Calyptratae, *Myiophorus doryphorae* (Family Tachinidae), *Ormia ochracea* (Family Tachinidae) and *Neobelliera (=Sarcophaga) bullata* (Family Sarcophagidae), and one representative of its sister taxonomic group the Acalyptratae, *Eurosta solidaginis* (Family Tephritidae, Fig. 1). The Calyptratae and the Acalyptratae are subsections of the Schizophora, which is a section in the sub-order Brachycera (McAlpine 1989). Of these four species, only one, *O. ochracea*, is known to be an acoustically orienting tympanate dipteran (Cade 1975; Walker 1986; Robert et al. 1992). The other three species are atympanate dipterans. Adult *O. ochracea* and *N. bullata* were obtained from laboratory colonies. *O. ochracea*, originally wild-caught from Gainesville, Fl., has been reared in our laboratory for more than 2 years according to the method of Wineriter and Walker (1990). Flies were maintained at 25.5±1 °C, 55%±3% relative humidity on a 16:8 light:dark regime. Adult *O. ochracea* were fed liquid hummingbird diet, whereas the larvae developed within the host cricket *G. bimaculatus*. Adults of another parasitoid tachinid fly *M. doryphorae* were obtained by collecting larvae of the Colorado potato beetle *Leptinotarsa decemlineata*. The beetle larvae were maintained on fresh potato leaves in the laboratory under the same environmental conditions as for rearing *O. ochracea* and allowed to pupate in a vermiculite-filled plastic container. The larvae of the parasitoid *M. doryphorae* pupated inside the beetle pupae and emerged from the vermiculite as adults. *Eurosta solidaginis* were collected in mid March as pupae overwintering in goldenrod galls from fields in Dryden, NY. The galls were incubated at 25.5±1 °C until adult flies emerged.

#### Scanning electron microscopy

The surface of the prothoracic region of females from each species was compared using scanning electron microscopy (SEM). Following dissection, isolated thoraces were dried, mounted on stubs and sputter-coated with gold using a Balzers sputter coater. Because of the larger size of the thorax of *N. bullata*, the posterior portion of the thorax had to be dissected away so that it would fit in the specimen chamber of the microscope. Specimens were viewed on a Hitachi 4500 scanning electron microscope and photographed with type 55 Polaroid film. For nomenclature of external structures we followed the terminology of Bonhag (1949).

#### Light microscopy

For-light microscope preparations, adult female flies at least 2 days old were fixed in alcoholic Bouin's solution (Pantin 1946;