NOTE

# Paralyzation and Developmental Delay of a Factitious Host by *Melittobia digitata* (Hymenoptera: Eulophidae)[1]

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Ectoparasites encounter at least two obstacles for successfully parasitization. Because they deposit their eggs on the outside of the host, the eggs may be dislodged if the host moves. Injection of a paralytic venom helps overcome this potential obstacle (Piek and Spanjer 1986. Pg. 161-307. *In* Venoms of the Hymenoptera: Biochemical, Pharmacological and Behavioral Aspects. Ed. T. Piek. Academic Press, London). Continued host development poses another obstacle, that of limiting the amount of time that the host is available for development of the parasite. Ectoparasitoids have, thus, evolved a variety of mechanisms involving developmental stasis or delay in host development (i.e., injection of specialized venom proteins, some of which influence host ecdysteroid production) (Marris et al. 2001. Physiol. Entomol. 26: 229-238).

*Melittobia digitata* Dahms (Hymenoptera: Eulophidae) is an ectoparasitoid that usually attacks the prepupae of solitary and, sometimes, social bees and wasps (Maeta and Yamane 1974. Bull. Tohoku Nat. Agric. Exp. Sta. 47: 115-131; Edwards and Pengelly 1966. Proc. Entomol. Soc. Wash. 96: 98-99). It is also a facultative hyperparasitoid, attacking both Hymenoptera and Diptera, such as bee flies of the genus *Anthrax,* that attack its habitual hosts (Krombein 1967. Trap-nesting Wasps and Bees: Life Histories, Nests, and Associates. Smithsonian Press, Washington, DC).

The objective of this study was to determine whether *M. digitata* is able to paralyze or halt development of a host. However, its most common host, the mud dauber *Trypoxylon politum* (Say), is virtually immobile in the prepupal and pupal stages, thus limiting its use for such determinations. Alternate, easily accessible and mobile hosts, useful for a bioassay for paralytic effects, are pupae of the yellow mealworm, *Tenebrio molitor* L. Preliminary tests showed that *M. digitata* would readily sting mealworm pupae.

Two hundred four newly-molted *T. molitor* pupae from a laboratory culture maintained at the University of Georgia Department of Entomology were placed individually in 1-dram glass vials (47 × 15 mm) with cotton stoppers. Five female *M. digitata*

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from a culture maintained in the laboratory were added to each of 102 vials containing mealworm pupae; the remaining 102 vials of pupae were kept as controls. All were maintained at ambient room temperatures (20 to 22°C).

After 48 h, *M. digitata* were removed, and all mealworm pupae were immediately tested for movement, using the 'gin-trap' reflex (Hinton 1946. Trans. Royal Entomol. Soc. Lond. 97: 473-96). This reflex occurs when a pupa, whose abdomen in a relaxed state, is somewhat decurved in relation to the thorax, suddenly straightens the abdomen, causing a set of sharp edges on the anterior and posterior margins of the medial tergites to come together. These structures are thought to help defend the pupa from small insects and mites (Crowson 1981. The Biology of the Coleoptera. Academic Press, London). To test for movement or mobility, each pupa was held individually by the thorax, with the thumb and forefinger of the investigator clasping its dorsal and ventral surfaces. With the forefinger and thumb of the other hand, the investigator gently squeezed the sides of the pupal head which invariably elicits abdominal twitching in unparasitized pupae. A second investigator, who was unaware of which larvae had been exposed to the wasps, performed a second test intended to elicit movement, in which the pupae were held as before and the abdominal tergites stroked, first with a small paint brush, then with a finger tip. In addition to mobility, we also recorded the number of pupae that metamorphosed into adults, and the number of visible dark spots on the pupae that appear at sting sites as noted by Malyshev (1968. Genesis of the Hymenoptera and the Phases of their Evolution. Edited Translation by O. W. Richards and B. Uvarov. Richard Clay [The Chaucer Press], Ltd., Bungay) using *M. acasta* (Walker).

The mean number of dark spots on pupae classified as immobile was 5.16 (SD = 3.41); the mean number of spots on pupae classified as mobile was 0.06 (SD = 0.24). A Mann-Whitney test showed that these means were significantly different (U = 113.00, df = 1, $P < 0.001$) (Statistica 6.0. StatSoft, Inc. Tulsa).

Estimations of mobility taken by the two investigators agreed 97.55% of the time. In the five cases in which only one investigator classified a pupa as mobile, the pupa was considered to be mobile for the purposes of this study. Mealworm pupae exposed to *M. digitata* were significantly less likely to move, with 68 out of 102 classified as immobile, compared to 3 out of 102 in the control group ($\chi^2 = 91.27$, df = 1, $P < 0.001$) (Statistica 6.0. StatSoft, Inc. Tulsa) (Table 1). A group of the immobile pupae observed 2d later under a dissecting microscope (40X) revealed internal movement of fluid that could be seen through the translucent pupal integument.

Significantly fewer mealworms developed to adulthood in the experimental group ($\chi^2 = 59.46$, df = 1, $P < 0.001$) (Statistica 6.0. StatSoft, Inc. Tulsa) (Table 2). Of the 68 mealworms in the experimental group that were classified as immobile, only one

**Table 1. *Tenebrio molitor* pupal movement after 48 h exposure to five female *M. digitata***

| Treatment | Movement evident | No movement |
|---|---|---|
| Female *M. digitata* present | 34 | 68 |
| Controls (female *M. digitata* absent) | 99 | 3 |

$P < 0.001$, $\chi^2 = 91.27$.

**Table 2. *Tenebrio molitor* pupal development after 48 h exposure to five female *M. digitata***

| Treatment | Developed to adult | Did not develop |
|---|---|---|
| Female *M. digitata* present | 26 | 76 |
| Controls (female *M. digitata* absent) | 81 | 21 |

$P < 0.001$, $\chi^2 = 59.46$.

eclosed as an adult. This was statistically compared with those that eclosed in the unparalyzed population of the meal worms exposed to *M. digitata* using a McNemar test (T = 18.27, df = 1, $P < 0.001$) (Statistica 6.0. StatSoft, Inc. Tulsa).

Our results confirm that *M. digitata* stings the host, as evidenced by the significantly greater number of dark spots on mealworm pupae that did not respond to stimuli, and that the substance injected by *M. digitata* affects both mobility and development of its host. The data support the hypothesis that the sting of *M. digitata* causes paralysis in mealworm pupae. It appears that the paralysis is caused by the injected venom, rather than physical trauma resulting from insertion of the sting. To test this hypothesis, it would be necessary to obtain and inject pure venom from the wasps, with corresponding injection of insect saline in controls. The reduction in mobility in mealworm pupae exposed to *M. digitata* could be due to death rather than to paralysis, but the observed internal fluid movement suggests that these hosts were simply paralyzed. Moreover, the immobilized pupae did not immediately decay or dessicate, but remained in an arrested state of development for an extended period without metamorphosing into adults.

Host paralysis following parasitization by chalcidoids has been reported in the aphelinid *Aphelinus jucundus* Gahan and the eulophids *Sympiesis viridula* (Thomson) (Parker and Smith 1933. Ann. Entomol. Soc. Amer. 26: 21-39) and *Dahlbominus fuscipennis* (Zettersted) (Clausen 1940. Entomophagous Insects. McGraw-Hill, NY). Coudron et al. (2000. Ann. Entomol. Soc. Amer. 93: 890-897) also arrested developmental activity in hosts injected with an aqueous extract from the venom gland of the eulophid *Necremnus breviramulus* Gahan.

Interruption of *T. molitor* pupal development by *M. digitata* sting components is strongly supported (Table 2). Although 20% of the controls failed to develop, it is possible that handling or other factors were detrimental to development. Because one of the 68 immobilized pupae developed into an adult, the possibility of a dosage-sensitive factor or factors that affect both paralysis and development exists. The ability to halt or delay development has been shown in at least two other species of ectoparasitic eulophids (Marris et al. 2001. Physiol. Entomol. 26: 229-238; Coudron and Brandt 1996. Toxicon 34: 1431-1441).

The *M. digitata*-mealworm interaction could also provide a useful classroom demonstration of how minuscule amounts of substances injected by a tiny wasp into a relatively enormous host can have dramatic effects on the host. Although the mealworm pupa is a factitious host, probably similar effects occur in normal hosts. Though less easily observed, these venom effects are no doubt of considerable adaptive significance in the parasitoid-host relationship.