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Variation in Propensity to Exhibit Thanatosis in *Nasonia vitripennis* (Hymenoptera: Pteromalidae)

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Thanatosis (death-feigning) has rarely been documented for Hymenoptera but occurs in the parasitoid wasp Nasonia vitripennis*. The propensity to exhibit thanatosis did not differ with age, sex, or food deprivation. Squeezing a female's abdomen and contacting her antennae were equally likely to trigger thanatosis. Dropping an object next to a female in order to cause substrate vibrations never triggered thanatosis, and dropping a female from a test tube rarely triggered thanatosis. Thanatosis was not seen during interactions between females. There was some tendency for females to exhibit fewer thanatosis responses on white than on colored backgrounds. Females that were least active had the greatest tendency to exhibit thanatosis.*

KEY WORDS: activity; death-feigning; *Nasonia*; parasitoid wasp; thanatosis.

INTRODUCTION

Thanatosis is also called death feigning or playing dead. Thanatosis has not been well-studied despite its occurrence in a wide variety of species, e.g., some species of mammals (Kimble, 1997), bird (Rovee *et al.*, 1976), snakes (Burghardt and Greene, 1988; Harding, 1997), amphibians (McCallum, 1999), fish (Gibran, 2004), spiders (Cloudsley-Thompson,

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1995), beetles (Chemsak and Linsley, 1970; Prohammer and Wade, 1981; Allen, 1990; Oliver, 1996; Acheampong and Mitchell, 1997; Miyatake, 2001a,b; Miyatake *et al.*, 2004), cicada (Villet, 1999), crickets (Nishino and Sakai, 1996 and references therein), stick insects (Godden, 1972; Carlberg, 1986), mantids (Edmunds, 1972), odonates (Abbott, 1926), and lepidopterans (Tojo *et al.*, 1985; Dudley, 1989; Larsen, 1991). Here we examine thanatosis in the parasitoid wasp *Nasonia vitripennis* Walker (Hymenoptera: Pteromalidae).

N. vitripennis is a parasitoid of the pupal stage of certain fly species that are found in association with carrion, refuse, or birds' nests (Whiting, 1967; Rueda and Axtell, 1985). Roughly 20–40 *N. vitripennis* develop per fly pupa, emerging as free living adults in about 2 weeks. Adults are a few millimeters long. They feed on honey in the laboratory and presumably nectar in the wild. Females also host feed. Females have full wings and fly, whereas males have short wings and do not fly.

N. vitripennis has a worldwide distribution (Rueda and Axtell, 1985) and is sold as a control agent for pest filth flies (Johnson, 1998). As insects go, it has been well-studied (e.g., references in Godfray, 1994). It is sometimes referred to as the *Drosophila* of the Hymenoptera (Pultz and Leaf, 2003), and it is a common textbook example of sex ratio behavior (Thornhill and Alcock, 1983; Krebs and Davies, 1993). However, there have been no previous studies of thanatosis in *N. vitripennis*, and thanatosis has not been well-documented in Hymenoptera. For example, a recent list of insect taxa exhibiting thanatosis does not include Hymenoptera (Miyatake, 2001b), and a book on parasitoids does not mention thanatosis (Godfray, 1994).

Thanatosis has been described as a stress induced seizure (e.g., Harding, 1997). In *N. vitripennis*, an individual falls onto its back, pulls in its limbs and antennae and becomes immobile for a few seconds before righting itself. Thanatosis is thought to serve an antipredator function, to serve as an alternative to running away (e.g., Rovee *et al.*, 1977; Prohammer and Wade, 1981; Miyatake, 2001b). Predators of adult *N. vitripennis* include spiders (Madej, 1992).

The goals of the present study were to examine potential sources of variation in propensity of *N. vitripennis* to exhibit thanatosis. (1) We examined sex differences. Propensity for thanatosis might differ between the sexes due to differences in life history that might affect their predation risks, e.g., females live longer, disperse farther and are larger. (2) We examined effects of food deprivation. Food deprivation may decrease the propensity to exhibit thanatosis by raising the cost of lost opportunity to feed that comes with being immobile (Miyatake, 2001a). (3) We examined physical triggers. (4) We examined effects of age. (5) We examined effects of

background color. Thanatosis might be less prevalent on light backgrounds than on dark: *N. vitripennis* are dark black-green and running away may be a more effective defense on light-colored backgrounds where color-match does not provide some degree of crypsis. (6) We examined effects of activity level. That prior activity level might influence thanatosis was suggested by observations during other experiments. (7) We examined interactions between conspecific females. That female interactions might elicit thanatosis seemed plausible given that females behave aggressively toward each other when defending hosts (King *et al.*, 1995). Furthermore, thanatosis has been observed occasionally in conspecific interactions in the confamilial *S. endius* (King unpublished).

METHODS

General Methods

Experiments were conducted with *N. vitripennis* from laboratory colonies of a scarlet-eyed strain. It was maintained on pupae of the blow fly *Calliphora vomitoria*, which were obtained commercially from GrubCo (Hamilton, OH) and stored in the refrigerator as pupae. The wasps were maintained in an incubator at about 26°C with a tray of water in the incubator to keep humidity high.

The males and females used for the first experiment were all isolated as pupae and thus were virgin. In contrast, the females for the other experiments were allowed to emerge from their hosts naturally (or in the fourth experiment the host was opened when the wasps were pupae so that wasp age could be determined), and mating was assumed to have occurred. Adult wasps were provided with honey unless specified otherwise. Thanatosis was recorded as occurring when the wasp flipped onto its back or side and retracted its legs.

Experiments

First, we tested whether males and females differed in their propensity to exhibit thanatosis. 30 males and 30 females that had emerged within the previous 3 days were tested by touching each wasp's antennae with the cotton covered tip of a 2 mm diameter stick. The antennae were touched up to five times, or until thanatosis was exhibited, whichever happened first. The frequency of individuals that did versus did not exhibit thanatosis was compared between the sexes with a chi-square test of independence.

Second, we examined the effect of food deprivation on thanatosis in females. Females were isolated singly in test tubes the day they emerged. Thirty females were given a small droplet of honey for food, and thirty were given no food at all. After 2 days with or without food, each female was tested as in the previous experiment. *N. vitripennis* live about 5 days without food (Girault and Sanders, 1910).

Third, we tested what physical stimuli trigger thanatosis in females. Females 1–7-day old were tested for thanatosis in one of four treatments. In the first three treatments, each female was allowed to walk out of her test tube onto a clean glass petri dish (10 cm diameter, 1.3 cm high); then either (1) her antennae were tapped once with metal forceps, (2) a large rubber stopper (5 cm diameter, 2.25 cm high) was dropped near the petri dish, or (3) her abdomen was lightly squeezed with forceps. In the fourth treatment, the female was tapped out of a clean test tube about six inches above the glass dish. Each wasp was treated with only one treatment, which was administered in three separate trials that were seconds apart. Whether the female went into thanatosis after the first, second, and/or third trial was recorded, so number of thanatosis responses could range from 0–3.

Fourth, we examined the effect of age on thanatosis in females. Females in the old treatment were 8–11-day old and females in the young treatment were 1–3-day old. Thanatosis was tested for each female by tapping her antennae with metal forceps in three separate trials as in the previous experiment.

Fifth, we examined whether the likelihood of exhibiting thanatosis was affected by background color. Each female was tapped out of a test tube onto a glass petri dish that was on top of either a brown, reddish brown, green, yellow or white piece of paper. She was allowed to acclimate for 5 min then tested for thanatosis as in the previous experiment.

Sixth, we tested whether the activity level of a female had any effect on the likelihood of her subsequently exhibiting thanatosis. Females were 0–10-day old at testing. First activity level was determined: a female was tapped out of her test tube into a deep plastic petri dish (8.5 cm diameter, 3.5 cm high), the dish was covered, and her locomotor activity was measured for 5 min. Locomotor activity was defined as time spent walking, hopping, or in flight (King *et al.*, 2000). Then thanatosis was tested for each female as in the previous experiment.

Seventh, we searched videotapes of female-female interactions for thanatosis ($n = 14$ pairs of females, each with 1 or 2 hosts, 6 h per pair; methods in King *et al.*, 1995).

RESULTS

The proportion of individuals exhibiting thanatosis did not differ significantly between males and females (17 of 30 versus 18 of 30; $\chi^2 = 0.069$, $df = 1$, $P = 0.79$) or between females with food versus without (16 of 30 versus 11 of 30) ($\chi^2 = 1.68$, $df = 1$, $P = 0.19$).

Dropping a stopper near a female never resulted in any thanatosis for the 63 females tested, and dropping her from a test tube resulted in at least one episode of thanatosis for only 5 of 65 females. In contrast, the other two triggers, contacting the antennae and squeezing the abdomen, were often effective in eliciting thanatosis. The effect of treatment was examined by *t*-test. Number of thanatosis responses did not differ significantly between females whose antennae had been contacted and females whose abdomen had been squeezed (1.1 ± 0.14 versus 0.84 ± 0.11; $t = 1.40$, $df = 131$, $P = 0.17$). Likewise, whether or not a female exhibited any thanatosis during her three trials was independent of treatment ($\chi^2 = 0.027$, $df = 1$, $P = 0.87$). After one trial, 27% of the 133 females exhibited thanatosis in these two effective treatments, within two trials 47% had and within three trials 59% had.

The proportion of individuals exhibiting thanatosis did not differ significantly between young and old females (41 of 52 versus 35 of 52; $\chi^2 = 1.76$, $df = 1$, $P = 0.79$) nor did number of thanatosis responses ($t = 0.48$, $df = 102$, $P = 0.64$).

The proportion of females that exhibited any thanatosis when disturbed was unaffected by being on a brown, reddish brown, green, yellow or white background ($\chi^2 = 4.68$, $df = 4$, $P = 0.27$). There also was no significant effect of background color when the number of thanatosis responses was analyzed across all colors (Kruskal Wallis test: $\chi^2 = 7.14$, $df = 4$, $P = 0.13$). However, an a posteriori test suggested some tendency for females to exhibit fewer thanatosis responses on white than on colored backgrounds (1.91, $n = 32$ versus 2.40, $n = 121$; Mann–Whitney $U = 1459.0$, $P = 0.014$).

Females that were least active had the greatest tendency to exhibit thanatosis (Fig. 1). Within one trial, 39% of the 96 females exhibited thanatosis, within two trials 49% had and within three trials 64% had.

Although females wing flicked, lunged at, and even grabbed and dragged the other female, they did not exhibit thanatosis in response to each other.

DISCUSSION

Neither sex nor age significantly affected the likelihood of exhibiting thanatosis in *N. vitripennis*. The effect of age appears not to have been

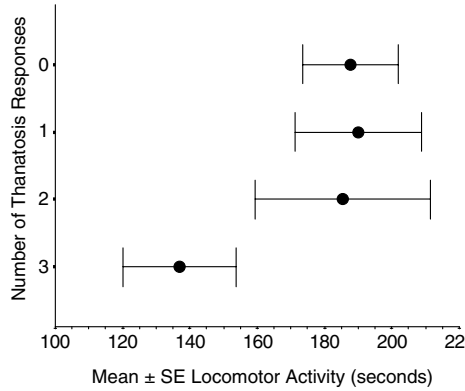

Fig. 1. Relationship between a female's locomotor activity (s active out of 300 s) and the number of thanatosis responses to three subsequent tappings of her antennae (Spearman rank correlation: $r_s = 0.21$, $n = 96$, $P = 0.043$).

examined in other insects, and the effect of sex has been mixed. Sweetpotato weevil females exhibit thanatosis more frequently than males under some conditions (Miyatake, 2001b); whereas in the red flour beetle and the cricket *Gryllus bimaculatus*, the duration of thanatosis does not differ between the sexes (Prohammer and Wade, 1981; Nishino and Sakai, 1996).

What is used to trigger thanatosis varies among studies, and most studies have not systematically tested different potential triggers as we did. Squeezing the thorax elicits thanatosis in the cricket *Gryllus bimaculatus* (Nishino and Sakai, 1996), similar to our eliciting it in *N. vitripennis* by squeezing the abdomen. A combination of contact and spilling out of a container elicits thanatosis in the red flour beetle and the sweetpotato weevil; however, whether both are necessary is not clear (Prohammer and Wade, 1981; Miyatake, 2001a,b). In the waterscorpion bug *Ranatra* sp., thanatosis can be elicited by being dropped or stroked or sometimes even just being picked up or having air blown on it or slight contact with the legs, abdomen or thorax (Holmes, 1906). Dropping a rubber stopper near females did not elicit thanatosis in *N. vitripennis*, whereas disturbing nearby live and artificial plants elicits "quiescence" in Colorado potato beetles (Acheampong and Mitchell, 1997). Plants probably make a better substrate for detecting vibration than do the more solid substrates on which *N. vitripennis* are found, and thanatosis may differ from quiescence (Holmes, 1906). *N. vitripennis* also exhibits "quiescence," where it stops moving but does not fall over and pull its limbs in as it does in thanatosis.

That less active individuals exhibited more thanatosis responses in *N. vitripennis* may be related to ease of transition. Both thanatosis and running away may allow escape from stressful situations; however, switching from inactivity to thanatosis may be an easier physical transition than switching from inactivity to running, whereas running away may be easier for individuals that are already in locomotion. Similar to *N. vitripennis*, sweetpotato weevils that are resting or feeding are more likely to exhibit thanatosis and stay in thanatosis longer than weevils that are walking (Miyatake, 2001b). In the cutworm *Spodoptera litura* the solitary phase is both less active and has longer episodes of thanatosis (Tojo *et al.*, 1985).

Whereas food deprivation had no significant effect on thanatosis of *N. vitripennis* females, it decreases the likelihood of thanatosis in sweetpotato weevils (Miyatake, 2001a). Miyatake (2001a) suggests that the advantages of continuing to search for food relative to escaping predation may be greater for food deprived sweetpotato weevils than for fed sweetpotato weevils. However, thanatosis lasts hundreds of seconds in the sweetpotato weevil versus only a few seconds in *N. vitripennis*. Thus, thanatosis may decrease opportunity to forage for sweetpotato weevils but not *N. vitripennis*.

This appears to be the first study specifically on thanatosis in any hymenopteran. How common thanatosis is in this order remains to be seen. Thanatosis also occurs in some other pteromalids, e.g., *Spalangia endius* and *S. cameroni* (personal observation) and in a bee (van Veen *et al.*, 1999).

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