

The effect of exposure to conspecifics on restlessness in the parasitoid wasp *Nasonia vitripennis* (Hymenoptera: Pteromalidae)

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Abstract—When habitat quality is variable, there should be strong selection for the ability to detect and respond to the variation. Adult females of the parasitoid wasp *Nasonia vitripennis* (Walker) are known to increase their restlessness (the proportion of time in locomotion) both during and after exposure to a poor quality host. Doing so provides a mechanism for leaving a poor host and potentially finding a better host. This study examined whether restlessness also changes in response to competition as indicated by the presence of adult conspecifics. Both restlessness and the probability of dispersing across an inhospitable environment were greater when a female was with another female than when she was alone. However, restlessness did not remain elevated after the other female was removed. In contrast with females, restlessness of males did not increase either during or after exposure to other males, and the probability of dispersing across an inhospitable environment was unaffected by the presence of another male. The difference between females and males may be related to differences in dispersal ability and in the abundance and distribution of hosts versus mates.

Résumé—Lorsque la qualité de l'habitat est variable, il devrait y avoir une forte sélection de la capacité de détecter cette variation et d'y réagir. Les femelles adultes de la guêpe parasitoïde *Nasonia vitripennis* (Walker) sont connues pour augmenter leur agitation (le pourcentage du temps consacré aux déplacements) au moment où elles sont mises en présence d'un hôte de faible qualité et durant la période qui suit. Cela leur procure un mécanisme pour abandonner un hôte peu intéressant et pour potentiellement en trouver un meilleur. La présente étude examine si l'agitation change aussi en réaction à la compétition marquée par la présence d'adultes de la même espèce. Lorsqu'une femelle est en présence d'une autre femelle, son agitation ainsi que la probabilité qu'elle traverse un environnement hostile sont plus grandes que lorsqu'elle est seule. Cependant, l'agitation accrue ne se prolonge pas lorsque l'autre femelle est retirée. Contrairement aux femelles, les mâles ne s'agitent pas plus durant ou après une rencontre avec d'autres mâles; leur probabilité de se déplacer vers un environnement hostile n'est pas affectée par la présence d'un autre mâle. La différence entre les femelles et les mâles peut être reliée à des différences dans leur pouvoir de dispersion et dans l'abondance et la répartition des hôtes plutôt que celles des partenaires.

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Introduction

Both habitats and locations within habitats may vary considerably in quality depending on variables such as resource availability, number of predators, and number of competitors. One of several mechanisms available for leaving poor habitats or locations is to increase restlessness (the proportion of time in locomotion) after exposure to poor conditions (King and Ellison 2006). This phenomenon has been documented in both migratory and nonmigratory species

(e.g., Caldwell 1974; Rankin and Riddiford 1977; Saks *et al.* 1988; Skalicki *et al.* 1988; Ashworth and Wall 1995; Keaser *et al.* 1996; Brzek and Konarzewski 2001). Increased restlessness can lead to dispersal on either a very large scale or a very small scale, depending on its duration. Poor conditions may affect walking and winged dispersal separately or may trigger just a general agitation. The present study examines restlessness, primarily in the form of walking, and dispersal across an inhospitable environment, using adults of a nonmigratory species,

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the parasitoid wasp *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae).

Adults of *N. vitripennis* are a few millimetres long. Males have short wings and do not fly, whereas females are macropterous and fly. Females oviposit more than one egg per host, typically 20–40. Hosts are the pupal stage of certain fly species that are found in association with carrion, refuse, or birds' nests (Whiting 1967; Rueda and Axtell 1985). The spatial distribution of hosts varies from highly clumped to just one or two hosts per patch (Werren 1983). The patches can be very far apart because the hosts are associated with carcasses, nests, and refuse piles. The offspring emerge as free-living adults in about 2 weeks, with males emerging before females.

The quality of hosts that a female encounters is known to affect her restlessness (King and Ellison 2006). For example, females are more restless after ovipositing in parasitized hosts than after ovipositing in unparasitized hosts. A parasitized host represents more competition: offspring from superparasitized hosts are smaller, have lower survivorship, and produce fewer offspring of their own (Wylie 1965, 1966). The present study tests, for both females and males, whether restlessness also changes in response to the direct presence of adult conspecific competitors and whether any changes in restlessness remain after the competitors are gone. Female parasitoid wasps are frequent subjects of optimal foraging studies, particularly studies of patch residence times, although relatively few studies have looked at the effects of the physical presence of conspecifics (reviewed in Wajnberg 2006).

Both female and male *N. vitripennis* sometimes exhibit intrasexual agonistic behavior (e.g., King *et al.* 1995). Females compete with other females for hosts as oviposition sites and as a source of fluids on which they themselves feed. Males compete with other males for females. Males stay near and defend hosts from which females may emerge and sites where they mated successfully (Altson 1920; King *et al.* 1969; van den Assem *et al.* 1980). Males may assess competition through responses to total density or the ratio of males to females (Saeki *et al.* 2005).

Methods

General methods

Nasonia vitripennis from laboratory colonies were maintained on pupae of the blow fly *Calliphora vomitoria* L. (Diptera: Calliphoridae;

obtained from Grubco, Inc., Hamilton, Ohio; allowed to pupate, and then refrigerated until needed). Except where noted, adult wasps were maintained on honey in group vials prior to use and were mated. Wasps were never reused.

Treatments differed in the number or sex of wasp(s) as described below. To determine the generality of outcomes, for each general question the conditions of exposure and the observer were varied among experiments. Conclusions were the same within a given general question despite having used different observers (compare experiments within each table (Tables 1–4)). Testing was for 10 min either during exposure to conspecifics or immediately after exposure in the absence of any conspecifics, depending on the experiment as noted below. Ten minutes was chosen to allow sufficient time for interactions but without habituation to each other. In treatments that contained multiple wasps, one wasp was randomly chosen for testing to avoid pseudoreplication.

Except in the two dispersal experiments, wasp restlessness was measured. Restlessness is the proportion of time that the wasp spent moving any distance by walking, hopping, or flying. Nonrestless wasps were standing still or grooming. Walking predominated restlessness. Hop and flight numbers were independently recorded but not analyzed statistically because few wasps hopped or flew during testing.

Within each experiment, treatments were blocked. Each block consisted of one of each treatment performed consecutively so that temperature, relative humidity, time of day, and wasp age were matched within blocks. Where the blocking effect (a correlation between treatments across blocks) was statistically significant, comparison of treatments was by a paired *t* test, or by a sign test when the assumption of normality was violated. When the blocking effect was nonsignificant, to maximize statistical power (Zar 1984), comparison was by independent *t* test or by Mann–Whitney *U* when the assumption of normality was not met. Normality was evaluated by *t* tests of skewness and kurtosis. Assumptions of equal variances were met. Because *t* tests are robust to deviations from their assumptions (Zar 1984), $\alpha = 0.001$ was used for tests of these assumptions, whereas $\alpha = 0.05$ was used for comparisons of means. Two-tailed *P* values are presented.

Table 1. Female behavior in the current female density experiments.

Treatment	<i>n</i>	Restlessness	No. of hops and flights
First experiment (no host, dish with damp sand)*			
One female	40	0.66±0.02 (0.25–0.88)	0.08±0.04 (0–1)
Two females	40	0.81±0.02 (0.36–0.94)	0.63±0.12 (0–2)
Second experiment (no host, glass vial)†			
One female	20	0.83±0.03 (0.42–0.97)	0.00±0.00 (0–0)
Two females	20	0.91±0.01 (0.73–1.00)	0.00±0.00 (0–0)

Note: Values in the table are means ± SE. Restlessness is the proportion of time in locomotion.

*Mann–Whitney $U = 218.0$, $P < 0.001$; medians (quartiles) 0.67 (0.60, 0.72), 0.81 (0.78, 0.87).

† $t_{38} = 2.08$, $P = 0.04$.

Table 2. Female behavior in the prior female density experiments.

Treatment	<i>n</i>	Restlessness	No. of hops and flights
First experiment (no host)*			
One female, 10 min	20	0.43±0.07 (0.00–0.95)	0.00±0.00 (0–0)
Two females, 10 min	20	0.52±0.07 (0.00–0.95)	0.00±0.00 (0–0)
Second experiment (1 host, 4 h)†			
One female	30	0.56±0.05 (0.00–0.94)	0.47±0.40 (0–12)
Four females	30	0.66±0.04 (0.30–0.97)	0.30±0.09 (0–1)
Third experiment (2 hosts, 1 d)‡			
One female	30	0.61±0.05 (0.17–0.98)	0.13±0.13 (0–4)
Four females	30	0.61±0.05 (0.12–0.99)	0.20±0.09 (0–2)

Note: Values in the table are means ± SE.

* $t_{19} = 1.44$, $P = 0.16$.

† $t_{58} = 1.60$, $P = 0.12$.

‡ $t_{58} = 0.44$, $P = 0.66$.

Female restlessness and dispersal

Current female density experiments

Two experiments using different containers examined the effect of current exposure to another female on restlessness. One container was a covered plastic dish (3.0 cm high, 8.3 cm diameter) with damp sand on the bottom half (the first experiment), and the other was a glass test tube (the second experiment). Either a lone female (treatment one) or two females (treatment two) were put in the container, and the restlessness of a single female was recorded. No hosts were present because females do not exhibit restlessness during oviposition.

Female dispersal experiment

This experiment also looked at the effects of current exposure to another female, but with the response variable being dispersal across an inhospitable environment, namely water (following King and Ellison 2006). Either one female or two females were placed on an island consisting

of an uncovered plastic dish (1.1 cm high, 3.4 cm diameter) filled with damp sand. This island was centred in another slightly wider uncovered dish (0.9 cm high, 5.6 cm diameter) half filled with water to create a moat approximately 1.1 cm wide. Individuals often appeared reluctant to launch and risked landing on the water. (Wasps landing on water stayed on the surface and eventually reached the other side.) This setup was designed to test motivation to disperse rather than to mimic natural conditions, although a similar situation could occur naturally. Whether the female left the island within 10 min and the number of nongrooming wing lifts prior to leaving were recorded.

Prior female density experiments

Three experiments addressed the effect of prior exposure to other females on the subsequent restlessness of a female. Testing occurred immediately after exposure in the absence of conspecifics. In the first experiment, each female was placed alone or with one other female

Table 3. Male behavior in the current male density experiments.

Treatment	<i>n</i>	Restlessness	No. of hops
First experiment (dish)*			
One male	39	0.57±0.06 (0.00–0.98)	0.03±0.03 (0–1)
Two males	39	0.54±0.06 (0.03–0.99)	0.03±0.03 (0–1)
Second experiment (dish with damp sand)†			
One male	42	0.59±0.04 (0.07–0.99)	0.00±0.00 (0–0)
Two males	42	0.56±0.04 (0.00–0.98)	0.00±0.00 (0–0)

Note: Values in the table are means ± SE.

* $t_{58} = 0.43$, $P = 0.67$.

† $t_{41} = 0.63$, $P = 0.53$.

Table 4. Male behavior in the prior exposure to conspecifics experiments.

Treatment	<i>n</i>	Restlessness	No. of hops
Prior exposure to males versus females experiment*			
One male to three females	20	0.90±0.03 (0.32–0.99)	0.00±0.00 (0–0)
One male to three males	20	0.89±0.02 (0.61–0.98)	0.00±0.00 (0–0)
Prior male density experiment†			
One male	30	0.69±0.03 (0.27–0.96)	0.00±0.00 (0–0)
Eight males	30	0.62±0.04 (0.21–0.96)	0.00±0.00 (0–0)
Prior male to female ratio experiment‡			
Four males to one female	30	0.86±0.04 (0.24–1.00)	0.00±0.00 (0–0)
One male to four females	30	0.88±0.03 (0.31–1.00)	0.00±0.00 (0–0)

Note: Values in the table are means ± SE.

*Sign test, $P = 1.00$; 10 positive differences and 10 negative differences.

† $t_{58} = 1.22$, $P = 0.23$.

‡Mann–Whitney $U = 445.5$, $P = 0.95$; medians (quartiles) 0.92 (0.83, 0.98), 0.94 (0.90, 0.97).

for 10 min in a plastic dish (3.0 cm high, 8.3 cm diameter, half-filled with damp sand, no hosts present), and then tested alone in the same container for 10 min. In the second experiment, prior to testing, one or four virgin females were given an unparasitized host for 4 h in a 20 mL glass vial (7.0 cm high, 2.0 cm diameter) plugged with cotton. Testing was in an empty glass terrarium covered with a sheet of glass. The third experiment was similar to the second except the duration of exposure to other females was 24 h, each vial contained two unparasitized hosts, and each female was isolated as a pupa in a test tube prior to use.

Male restlessness and dispersal

Current male density experiments

Two experiments examined the effect of current exposure to another male. Either a lone male or two males were placed in an empty plastic dish (3.0 cm high, 8.3 cm diameter), and the restlessness of a single male was recorded. The second experiment was similar except the dish contained damp sand (matching conditions

under which restlessness was most affected by density for females (see Results)).

Male dispersal experiment

This experiment matched that of the female dispersal experiment (see earlier) and was included because, although males do not fly, they were still able to leave the island by moving their legs on the water.

Prior exposure to conspecifics experiments

The remaining three experiments addressed the effect of prior exposure to conspecifics on male restlessness. The first experiment (prior exposure to males versus females) addressed the effect on male restlessness of previous exposure to males or females, controlled for total wasp density. Prior to testing, one virgin male and three virgin females (treatment one) or four virgin males (treatment two) were placed together in a test tube plugged with cotton. Time to mating was recorded in treatment one and used as the time interval that males were left with their conspecifics for both treatments. Thus, these data were analyzed by a sign test

(assumptions for a paired *t* test were not met), matching the two treatments according to the duration the males had been with their conspecifics.

The second experiment (prior male density) addressed the effect of previous exposure to other males. For the 30 min prior to testing, a virgin male was either alone or with seven other virgin males in a test tube plugged with cotton.

The third experiment (prior male to female ratio) addressed the effect of high versus low competition for mates, controlled for total wasp density. Treatments consisted of five virgin wasps in either a 4:1 or 1:4 sex ratio, placed together in a test tube plugged with cotton for the 10 min prior to testing.

Results

Females were more restless while in the presence of another female (Table 1). Females were also more likely to leave the island in the presence of another female (73% of females that were with another female dispersed within 10 min versus only 43% of lone females; likelihood ratio test $\chi_1^2 = 5.65$, $P = 0.017$). The number of wing lifts did not differ significantly between treatments ($t_{58} = 0.23$, $P = 0.82$; mean \pm SE = 1.45 ± 0.21 across treatments, range = 0–8). However, restlessness of females did not remain elevated once the female(s) they had been with were no longer present, regardless of the specific number of females, the absence or presence of hosts, and the duration of exposure to hosts (Table 2).

Male restlessness was not significantly affected by treatment in any of the male experiments, *i.e.*, by currently being alone versus with another male or by previous exposure to other males, males instead of females, or a higher ratio of males to females (Tables 3, 4). Likewise, current exposure to another male had no significant effect on how quickly males left the island (20% of males that were with another male dispersed within 10 min versus 15% of lone males; likelihood ratio test $\chi_1^2 = 0.17$, $P = 0.68$). The number of wing-lift episodes did not differ significantly between treatments ($U = 180.0$, $P = 0.60$; mean \pm SE = 1.18 ± 0.28 across treatments, $n = 40$, range = 0–7).

Discussion

The effect of conspecifics on restlessness differed for females versus males. Competition in the form of being with a conspecific female

increased the restlessness and dispersal of *N. vitripennis* females. Increased dispersal in the presence of other females also occurs in another pteromalid parasitoid, *Pachycrepoideus vindemiae* Rondani (Goubault *et al.* 2005). How this relates to current optimality models is not clear cut, and Goubault *et al.* (2005) call for the development of additional models. However, restlessness in the presence of a conspecific female should be adaptive in *N. vitripennis* if leaving the immediate vicinity of another female (which is not necessarily the same as leaving an entire patch of hosts) increases her probability of encountering a host that is not already parasitized or in the process of being parasitized. When females left the island, they often went just a little past the water and then walked around. The hosts of *N. vitripennis* are often clumped, sometimes with 50 or even thousands of hosts per patch, and often only a small proportion of these are parasitized (Werren 1983), so a female that leaves the immediate vicinity of another female may well encounter an unparasitized host.

The increased restlessness of females appears to be short-lived: it was not detected once the other female was gone. Prior exposure to a conspecific female also has no significant effect on either the duration of tethered flight in *N. vitripennis* females or the propensity to exhibit it (King 1993). In another pteromalid parasitoid, *P. vindemiae*, prior exposure increases the proportion of females that leave within 15 min, although females that remain stay longer (Goubault *et al.* 2005). In a scelionid, *Trissolcus plautiae* (Watanabe), prior exposure causes most females to stay longer (Ohno 1999). Prior exposure has no effect on patch residence time in *Leptopilina heterotoma* (Thomson), a figitid (Visser *et al.* 1992).

In contrast with females, there was no evidence that current or prior exposure to conspecifics or competition for mates affects restlessness in *N. vitripennis* males. Why do *N. vitripennis* males, unlike females, not become restless in the presence of a potential competitor? Under natural conditions, a male being more restless would likely remove himself not only from other males, but also from females. Males emerge first and wait for females to emerge (Whiting 1967). If males leave, they will be leaving a site from which females will soon emerge. After mating, females fly off in search of hosts. Because males cannot fly, the best opportunity for mating is probably at the natal site, despite the presence of other males. Staying despite the presence of other males is probably not beneficial in all species. For

example, in Roesel's bush cricket, *Metrioptera roeseli* (Hagenbach) (Orthoptera: Tettigoniidae), males move faster and farther away in the presence of another male, apparently to avoid acoustic interference when they call for mates (Berggren 2005).

Prior exposure to ovipositing females and their hosts did not affect restlessness in the present study, whereas prior exposure to parasitized hosts increased restlessness of females in an earlier study (King and Ellison 2006). This difference may be explained by the different levels of competition and thus the adaptive value of leaving in search of new hosts. In the respective studies, the offspring inside the hosts were in different stages of development, recently oviposited eggs versus larvae. Eggs are less competition for a female's offspring because they have not consumed part of the host and will be slower to do so than older larvae. Females are more likely to parasitize hosts with younger competitors (Werren 1984) and because of this may be less motivated to disperse when they encounter them.

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