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ABSTRACT:

Two main areas of focus were examined in three experiments: the effects of mating status on male activity and the effects of crowding on female activity in the parasitoid wasp *Nasonia vitripennis*. The first experiment dealt with wing dispersal among lone versus crowded females. Competition appeared to make the females more likely to wing-disperse than when the female was alone. The second experiment dealt with the locomotor activity of lone versus crowded females. Competition appeared to cause females to be more active when with another female than when alone. The final experiment dealt with the locomotor activity of mated versus virgin males when exposed to a dead virgin female. Mated and virgin males appeared to be equally active.

Abstract:

Two main areas of focus were examined in three experiments: the effects of mating status on male activity and the effects of crowding on female activity in the parasitoid wasp *Nasonia vitripennis*. The first experiment dealt with wing dispersal among lone versus crowded females. Crowding appeared to make the females more likely to wing-disperse than when the female was alone. The second experiment dealt with the locomotor activity of lone versus crowded females. Crowding appeared to cause females to be more active when with another female than when alone. The final experiment dealt with the locomotor activity of mated versus virgin males when exposed to a dead virgin female. Mated and virgin males appeared to be equally active.

Introduction:

Nasonia vitripennis is a parasitic wasp. The females oviposit in the pupal stage of fly species that are associated with decaying organic matter, animal carcasses, e.g. The wasp larvae feed on the fly pupae. When done feeding, they emerge from the host after about fourteen days at 25 °C. The females emerge fully winged and able to fly. They commonly mate, then disperse to find new patches of hosts (Shuker and West 2004). Dispersal to new patches is important because the original patch may no longer contain suitable hosts for oviposition (King et al. 2000). Dispersal may also occur due to poor environmental conditions (King 1993). Another situation where it may be advantageous for females to disperse is when other females are present. Their presence indicates a higher risk of competition for a female's offspring. Dispersing could increase the chance of finding a lower competition site. The first two experiments will examine the effects of crowding on a female's tendency to wing-disperse or have a higher activity level.

The first experiment compared time it took for crowded females to wing disperse versus time it took for lone females to wing disperse. The second experiment compared the locomotor activity of lone versus crowded females.

In another species of parasitoid wasp, *Spalangia endius,* virgin males tend to have a higher activity level than mated males (King et al 2005). The reason for mated males being stiller seems to be that they are busy replenishing their sperm supplies for subsequent matings (Fischer & King unpublished material). The third experiment tested for the activity difference in *Nasonia vitripennis.* Locomotor activity was compared between mated and virgin males when exposed to a dead virgin female.

Material and Methods

General Methods

The *N. vitripennis* were from laboratory colonies in test tubes. Water and honey were provided in each test tube for nourishment after emerging from the hosts. The test tubes were kept in a 25 °C incubator. Wasps were used form the same oviposition day to eliminate variation in locomotor activity caused by age.

Experiment One

The *N. vitripennis* were from laboratory colonies in test tubes. A water and honey mixture was provided in each test tube for nourishment after emerging from the hosts. The test tubes were kept in a 25 °C incubator.

Females were taken out of the main test tubes and placed in groups of one or two. Each group was placed in a separate test tube containing both water and honey. They were then left in the tubes for at least five minutes to feed, and thus rule out differences between treatments in hunger or thirst. Females were 14 to 22 days old.

Either a lone female or two females were placed on an island, an uncovered small petri dish that had been filled with sand that had been wetted. This small dish was centered in a medium-sized petri dish that was half full of water. When placed in this setup, females walk around and around the periphery of the inner dish. Occasionally they face outward and lift their wings as if about to fly, but they often then put their wings down again and resume walking around the periphery. This may happen repeatedly. Eventually, they may launch themselves out across the pool of water, but they risk landing in the water, from which it is difficult for them to extract themselves. This experiment was designed to test motivation rather than to mimic natural conditions; however, they might encounter a similar situation after heavy rains. In the two female treatment, a single (focal) female, randomly chosen, was followed. Using a stop watch, how long until the female left contact with the cap was recorded (up to 10 minutes). The number of wing-lift episodes was also recorded. To count as a new episode, there had to be at least two seconds between episodes. Lifting of wings to groom was excluded. New test tubes, petri dishes, and sand were used for each trial. The date, time, temperature, relative humidity, and oviposition day were also recorded and examined for differences. 30 replicates of each treatment were examined (King unpublished material).

Experiment Two

For each trial, two treatments were examined (n=20 trials). The first treatment was a lone female placed in a test tube, and the second treatment was two females together in another test tube. For each treatment, the movement time of the wasps was recorded for a ten minute interval. Movement time included walking, hopping, or flying. Activities such as standing still, grooming, or fighting were not recorded as locomotor activity. For treatment two, the wasp that was observed and recorded was chosen randomly. New test tubes were used for each trial.

The date, temperature, oviposition day, and relative humidity were also recorded. The temperature ranged from 23 ° C to 25 °C and the relative humidity ranged from 11% to 30 %. The relative humidity range was large; however, it did not affect the activity in the treatment with the lone female(Spearman rank correlation = 0.27, n = 20, 2tP = 0.25) or in the treatment with the crowded females (Spearman rank correlation = 0.26, n = 20, 2tP = 0.91).

Experiment Three

Pairs of a virgin and a mated male were videotaped together in the presence of a dead virgin female. The dead virgin female had emerged and been frozen the previous day. For testing the males, she was placed in a small petri dish (3.4 cm diameter by 1.1 cm high) filled about three-fourths of the way with off-white sand that had been wetted with water to keep the wasps from burrowing and to keep the humidity high, which reduces static. She was placed near the edge, dorsal side up. A glass cover was placed on the dish. A clean dish and cover and new wasps were used for each test. A live mated male and live virgin male were tapped out of their test tubes into the dish, opposite the female. Which male was put in first was alternated and recorded. Locomotor activity date were collected from the videotapes by watching the tapes in real time and using a stopwatch. Data were collected on the time spent in locomotion prior to the first male mounting the female (King unpublished material).

Results:

Experiment One

The single females took statistically longer than the double female treatments before wing-dispersing (434.83 sec. +/- 43.73 s.e. vs. 290.49 sec +/- 41.92 s.e.; $t = 2.38$, $df = 58$, $p = 0.02$). However, the results showed that there was no statistical difference between the single female versus the double female treatments in terms of number of wing-lift episodes (1.50 +/- 0.26 vs. 1.40 +/- 0.34; $t = 0.23$, $df = 58$, $p = 0.82$). There was no significant relationship between dispersal time and time of day, temperature, relative humidity, or oviposition day. The proportion of females that never dispersed was 57 % in the single female treatment versus 27% in the double female treatment.

Experiment Two

The crowded females showed more locomotor activity than lone females (544.47 +/- 8.94; 500.72 +/- 19.05; $t= 2.08$, $df=38$, $2tP=0.04$). The minimum and maximum time spent in locomotion for the lone female treatment was less than the double female treatment (Table 1).

Table 1 : Time (s) spent locomoting by single vs. paired females.

Treatment	Mean	N	SE	Minimum	Maximum	Kurtosis	SE of Kurtosis	Skewness	SE of Skewness
1 female	500.7240	20	19.04941	253.29	581.73	2.296	.992	-1.509	.512
2 females	544.4685	20	8.93683	435.85	598.63	1.737	.992	-1.319	.512
Total	522.5963	40	10.95971	253.29	598.63	4.792	.733	-1.982	.374

<u>**Experiment Three**</u>

This experiment showed that mated males and virgin males did not differ in time spent active when exposed to a dead virgin female (Sign test, n = 52, 2tP = 0.49; Table 2). There was only about three seconds difference in total locomotion time between the two treatments (Table 2).

Table 2 : The time (s) that virgin vs mated males spent in locomotion.

	N	Minimum	Maximum	Mean	SE
Virgin males	52	7.19	523.91	206.4473	20.5617
Mated males	52	8.13	493.72	202.9788	19.4192
Difference	52	-305.32	145.49	3.4685	9.5943

There was a tendency for the difference in activity between virgin and mated males to be greater when there was no attempt to mate the dead female, but the difference was not statistically significant (Mann-Whitney U = 179, n_1 = 38, n_2 = 14, 2tP = 0.073).

Discussion:

Experiments One and Two

Crowded females appear to have a higher tendency to wing-disperse and to have a higher activity level than single females. This shows that competition from another female has an effect on locomotion. Having two females together on the same petri dish seems to make them eager to leave; it even makes them willing to risk flying over a harsh environment in experiment one. However, no significant difference was observed in wing-lift episodes between the two experimental treatments in experiment one. This shows that although crowded females are more likely to disperse and move around; they do not differ in the number of intention movements prior to dispersing.

Although differences between treatments were controlled, there were a few minor differences that may have contributed to the difference in activity or dispersal. One aspect that was difficult to control was the amount of handling the wasps received. Some wasps were harder to get out of the test tubes, so they received more taps on the tube. This may have shaken them up and contributed to their activity level.

In a previous study, many treatments were examined to test what affects tethered flight in *N. vitripennis* females, including a crowding experiment similar to experiment one except comparing lone females to females that had been in groups of ten (King 1993). Exposure to many other females did not affect duration of flight activity or number that flew at all when compared to a single female. Thus, method of testing appears to affect how crowding affects flight.

One study has examined the flight activity of *N. vitripennis* when exposed to an unsuitable host versus when exposed to a suitable host. Wasps were placed in the same set up as experiment one (on a small petri dish filled with sand centered in a medium petri dish half-full of water). There was no significant difference between dispersal time or wing-lifts between the two treatments; therefore, being exposed to a long-dead host was not sufficient motivation for the wasps to wing-disperse over water (Ellison and King 2005). Thus some but not all aspects of enviromental quality affect flight dispersal.

Experiment Three

When exposed to a dead virgin female, *N. vitripennis* mated and virgin males seem to have equal activity levels. This behavior is in direct contrast with the behavior of another wasp species, *Spalangia endius* (King et al. 2000). Virgin males of *S. endius* show a higher level of activity than mated males, usually resulting in the virgin males mating the female first. This shows that mating does not have as much of an effect on male locomotion tendencies of *N. vitripennis* as it does on *S. endius*. Interacting with a mated female apperas to be an unpleasant experience for male *S. endius* (King et al. 2000), whereas not in *N. vitripennis* (King unpublished data). This may explain the reduced activity of *S. endius*, but not *N. vitripennis* males after mating.

In a previous study, the locomotor activity was compared between mated and virgin females of *N. vitripennis*. The results from this study showed that mated females were more active than virgin females up to two hours after mating. The results were shown to be independent of head width and did not affect a female's subsequent offspring production (Grimm, King, and Reno 2000). It appears that mating status has more of an effect on female *N. vitripennis* than male *N. vitripennis*.

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