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NORTHERN ILLINOIS UNIVERSITY

Mating Behaviors in Parasitic Wasps

A thesis submitted to the university honors program in partial fulfillment of the requirements for the baccalaureate degree with upper division honors

Department of Biology

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ABSTRACT

Mating behavior was examined in two species of parasitic wasps, *Spalangia endius* and *Nasonia vitripennis*. Contact, mounting, courting, and copulatory behavior were investigated for both species. Observations over reencounter behaviors were also examined. Male mate choice of virgin or mated females was looked at for *N. vitripennis*. *N. vitripennis* tended to initially contact both virgins and mated females. However, males copulated more with virgins. The behaviors that showed up most consistently in both species were wing fanning, leg tapping, and dorsal mounting. When the wasps would reencounter each other after copulation, *S. endius* would not court again, whereas *N. vitripennis* will. These females even became receptive to copulate again.

INTRODUCTION

Studying mating behavior is important for the understanding of mate choice and mating systems. Mating behavior is probably best studied and understood in vertebrates such as chimpanzees. This study examined two species of parasitic wasps, *Spalangia endius* and *Nasonia vitripennis.* Mating behavior has not been well studied in insects, especially in parasitic wasps. Findings of this experiment can be compared to other species of wasps to give a general overview of mating behavior in this group. It may also be beneficial in our understanding of the evolution of mating behavior. Understanding parasitic wasps is important because they are speciose with >100,000 species, many of which are important in controlling pest insects (van den Assem, 1986). More aspects of courting and mating behavior are understood in *N. vitripennis* than in *S. endius* since many studies have been conducted on this species. *N. vitripennis* seems to have become a model system for research in evolution and ecology (van den Assem et al, 1994). *S. endius* has not been used for many studies, therefore, almost no data has been published on its mating behavior. This seems surprising since the wasps is cosmopolitan and common in habitats people frequent.

Both species of these wasps share similar characteristics. Both are parasitic on the pupae of several species of flies (King, 2002). The adult female wasps lay eggs within the hardened larval skin of the puparium (Carolina Biological Supply Company (CBSC), 1995). The eggs are small, white, and pear shaped. The average development time for *N. vitripennis* is about two weeks, broken down as follows: eggs, two days; larva, 6 days, pupa, 6 days (CBSC, 1995). *S. endius* takes about a week longer. Throughout the development, the offspring feed on the host, killing it in the process (King et al, 2000). After the feeding is complete, the adult wasps chew through the puparial wall and emerges. Both of these species are haplodiploid, where unmated females can only produce males, whereas mated females can produce both females and males (CBSC, 1995).

Characteristics such as mating behavior are different among the two species. The amount of eggs a female deposits into the host pupae differs as well. *S. endius* only deposits one egg per host, while *N. vitripennis* deposits about 20 eggs on a host (King, 2002). The flies that *S. endius* live in are frequently found in manure or rotting vegetation. These flies include houseflies and stable flies, both pest species. *N. vitripennis* lives in the eggs of blow flies, which are found around dead animals (Rueda et al, 1985).

Here I describe aspects of mating in *S. endius*. The data was collected from videos so I could see detail in behaviors that happen very quickly. In *N. vitripennis*, I tested whether males show choosiness in female mate selection. Despite *N. vitripennis* being well-studied, this aspect has not been examined before.

METHODS AND MATERIALS

Spalangia endius

A videotape of 22 separate pairs of virgin male-female pairs mating was observed. This video was made a previous semester by a graduate student. I observed and recorded data about various behaviors under four main categories: premounting, postmounting precopulatory, postcopulatory, and reencounter behaviors. I then tallied up the numbers for each specific behavior looked at. I was interested not just in what behaviors occur but also whether all pairs do the same.

Nasonia vitripennis

A small petri dish (diameter of 3.5 mm) with ¾ full of wetted sand was used for the experiment with *N. vitripennis*. A virgin female, mated female, and a mated male were all placed in the dish and covered with a glass lid. Mated males and females that had never interacted with each other were used. Which female the male contacted, courted, mounted, and copulated with first was observed. Time was kept with a stopwatch, and I recorded the amount of time it took until the wasps mated. If the time reached ten minutes or if mating occurred, the experiment was ended. 125 trios of wasps were used for this experiment.

Each trial of the experiment had new wasps, a disinfected dish, glass cover, and sand. The table was wiped down with bleach and then water to remove any pheromones the previously tested wasps may have left behind because the next wasps tested might be affected by being attracted to the scent. In every other trial, the virgin and mated female were put in the dish on different sides in case the male prefers one side over the other.

Prior to the experiment, males and females were separated in their pupal stage to insure they remain virgins. When mated males and females were needed, a virgin male and female were put in a tube together to mate. A couple of these were set up at a time. The female from one tube and a male from another were used for one trial and vice versa for the next.

At the start of each session, the temperature, relative humidity, the female age, and the male age were recorded. The mean temperature for throughout the entire experiment was 21.42 Celsius, range 20-22, n=125. The relative humidity had a mean of 46.41, range 32-60, n=125. The male age averaged 1.02 days old, range 0d-2d, n=125.

The female age averaged 1.01 days old, range 0d-2d, n=125. After the completion of the experiment, the data were analyzed using the computer software, SPSS.

RESULTS

Spalangia endius

Out of the 22 pairs, two of them did not mount. They did not even show premounting behaviors. I later found out that these two pairs had mated females. The data below consists of the 20 pairs that did demonstrate mating behavior. The behavior looked at is shown on the left. The number of wasps that demonstrated that behavior is tallied under yes, those that did not are under no, and those that were difficult to see in the video are listed in the unclear column.

Particular behaviors were observed in all 20 pairs (Tables 1A-D). Behaviors that were always observed in all of the pairs were dorsal mount of female, male's body vibrating, male's head above the female's thorax, male's antennae on either side of female, the male on the back and to the side to copulate, female's antennae extended after copulation, and the male back off of the female. Such behaviors as swaying side to side, female tapping antennae, antennae touching the female's head, wings moving, and grooming immediately after copulation were never observed in any of the 20 pairs. Behaviors that occurred in some pairs but not all were males fanning their wings, female extending her antennae during courtship, female's ovipositor popping out, male's antennae touching female's abdomen, male's forelegs on female's thorax, and males tapping their legs as they dismount.

Nasonia vitripennis

Males' initial choice of which female to contact and mount tended to be fairly equal (Table 2A). However, males copulated more with virgin females about 91% of the time (Table 2A). The female the male contacted first, influenced who he mounted ($X^2 = 90.64$, df = 1, 2tP < 0.001). The majority of the time, when the virgin female was contacted first, she was also mounted first. Likewise when the mated female was initially contacted, she was also mounted first (Table 2B). When the virgin was mounted first, then she was almost always the first to copulate with the male (Table 2C). When the mated female was mounted first, the male only copulated with her first half of the time ($X^2 = 40.72$, df = 2, 2tP < 0.001).

Factors that may have contributed to results.

Means are presented as mean +/- standard error.

Relative humidity did not differ between males that first mounted the virgin versus the mated female (46.66 +/- 1.22 versus 46.16 +/- 1.16; t = 0.298, df = 123, 2tP = 0.766). Relative humidity also did not differ between males that copulated the virgin versus the mated female (46.25 +/- 0.96 versus 50.04 +/- 1.84; t = 0.298, df = 123, 2tP = 0.766). However, relative humidity was lower with the 10 males that did not copulate (39.10 +/- 2.57; t = 0.298, df = 123, 2tP = 0.766). The mating process also took longer when the relative humidity of the room was lower (Fig. 1).

There was no difference in the male's age whether he mated with the virgin female, mated female, or none (1.08 days since emerging as an adult vs. 0.85 days vs.

0.95 days). The female's age did not differ either whether the male mated with a virgin female, mated female, or none (1.07 vs. 0.83 vs. 0.95).

Overall time for mating

A difference was found in the amount of time it took for the male to make contact. Males that contacted the virgin female first did so sooner versus those that contacted the mated female first (99.19 +/- 14.40 sec. versus 248.82 +/- 23.46 sec.; t = 5.351, df = 120, 2tP < 0.001). It took less time for males that mounted the virgin versus the mated female first (87.92 +/- 9.69 sec. versus 259.41 +/- 24.33 sec,; t = -6.430, df = 121, 2tP < 0.001). The opposite was found for the time it took for males to copulate. It took longer to mate for males that first mated with the virgin versus the mated female (150.16 +/- 13.47 sec. versus 93.92 +/- 14.01 sec.; t = 2.083, df = 111, 2tP = 0.040).

DISCUSSION

Spalangia endius

Premounting behavior

Prior to mounting the majority of the males fanned their wings suggesting that it may play a role in stimulating the female or drawing female odor to the male. As a comparison, *Spalangia cameroni*, which also produce one offspring per host, also fan their wings as they approach the female (King, 2000). None of the *S. endius* males were observed swaying side to side and most of the females did not have their antennae tucked in. Therefore, these behaviors are most likely not necessary in this species prior to mounting. Whether *S. cameroni* sways side to side is not known.

Postmounting, precoupulatory behavior

Many behaviors were looked for during this phase of mating to learn what behaviors are common during this time and what behaviors can entice the female into copulating with the male. As the males mounted the females, a little over half of them were seen touching the female's abdomen with their antennae. There may have been more males demonstrating this behavior, but the video was unclear for six of the pairs. Males may be able to tell at this point if the female has mated already. In the video with the mated females, the male would back away and never mount the female once his antennae touched her abdomen. In addition, during the reencounter, the male walked away in most cases. Pheromones secreted from the abdomen after copulation may be an explanation for this phenomenon. This will be further discussed under reencounter behaviors.

The males were always observed mounting the female dorsally. However, there may be a disadvantage to dorsal courting. Scientists have observed that when the male induces receptivity in the female from his courting, another male may sneak in and copulate with her before the male backs up (van den Assem, 1986).

Most of the time in this study, the females had their antennae extended and they never tapped them. The extended, non-tapping antennae are also seen in females of *S. cameroni*. This may suggest that antennae pulling in have no function in signaling the male that she is receptive since the female would still become receptive. In contrast, the male always vibrated his whole body and tapped his middle legs, which may help to entice the female. All of these behaviors my cause the female to become receptive, as evidenced by the female's genital orifice opening. Perhaps without these cues, she would not respond.

S. cameroni males also tapped their middle legs but on the female's thorax. However, unlike *S. endius*, the males of *S. cameroni* have also been observed to tap their antennae on the female's head (King, 2000). This antennal tapping, punctuated with pauses rather than being continuous, would also move up and down in synchrony (King, 2000).

Postcopulatory behavior

The customary behaviors of this species after copulation, is that males, as well as *S. cameroni* males, always returned to his precoupulatory position, leg tapped as he did before copulation, backed off the female, and tapped their legs while dismounting her (King, 2000). Once off the female, males were not observed to continue with their leg tapping. None of the wasps were seen to groom themselves immediately after copulation. Postcopulatory courtship that is similar to precoupulatory courtship is seen in other parasitic wasp species (van den Assem, 1986). Male wasps may perform postcopulatory courtship to decrease sperm competition by ensuring no other male copulating with the female before she is inseminated.

Reencounter behavior

Other species of wasps, such as *N. vitripennis* and *S. cameroni*, are observed to mate with the same partner again after already copulating once. However, this behavior was not seen in this species. When the male and female reencountered each other, most males would walk away after feeling the female's abdomen. As suggested earlier, females may release pheromones after mating, allowing the male to know if the female has previously mated. This can be advantageous for the males because of mate competition. Since males seem to not want to mate with mated females, the original male that contributed the sperm will benefit because his sperm will not be removed by subsequent males and his genes will be passed along.

One of the males did start to remount the female, but the female did not become receptive. Past studies demonstrate that *S. cameroni* mates repeatedly with no lag time: males mount, court, and try to copulate with virgin or previously mated females. Attempted copulations with females the male just successfully mated are also observed in *S. cameroni* (King, 2000). However, most of the time when a male mounts a mated female, the female begins walking and stroking her hind legs over her abdomen, as if trying to brush off the male (King, 2000). This brush off behavior has been observed in both *S. endius* and *S. cameroni.*

It seems unclear why in some species, female wasps will become receptive to another male after she already copulated with another male. In *S. endius*, where females are not receptive once they have been inseminated, it may be an advantage to the male. This reduces the probability of a second copulation, which would lead to sperm competition (van den Assem, 1986). A study has looked at the reencounter behavior of *N. vitripennis* since the females will copulate after insemination. The second male never inseminated successfully if he copulated soon after the first male. From an evolutionary adaptive standpoint, this does not seem to benefit anyone. By this second male copulating with a mated female, he is expending time and energy. However, the second male's sperm was utilized when the second copulation was 24 hours or more after the first (van den Assem, 1986). However, there was still sperm competition in that the second male's sperm mixed with the first male, therefore, the female had daughters that could have been sired by either male (van den Assem et al, 1976).

Nasonia vitripennis

As seen in *S. endius*, particular behaviors during courtship and mating were observed while performing the experiment with *N. vitripennis*. Scientists have noticed motor patterns in the movement of the head, antennae, mouthparts, wings, and legs (van den Assem et al, 1994). The antennal sweeps and the nodding of the head are conspicuous (van den Assem et al, 1994). *N. vitripennis* always courted dorsally on top of the female with the male's head being above the female's head and tapped his front legs. This display is thought to produce stimuli that temporarily immobilize the female and make her receptive (van den Assem et al, 1994). The bristles on the legs are thought to be the specific tactile stimulus that induces receptivity (van den Assem, 1986). The receptive female invites the male to copulate when she raises her abdomen to expose her genital orifice. Van den Assem claims that the female's antennal movement signals onset of receptivity to the mounted male and elicits his backing up to copulate (van den Assem et al, 1994). Van den Assem noticed that the female would lower the antennae and draw them tightly against her head at the onset of receptivity at which the male would then back up to copulate (van den Assem, 1986). As seen in both experiments that I preformed, the male reassumes his precopulatory position after copulation and before dismounting.

The movements of the mouthparts in both species of wasps were not noted by me since they were so difficult to see. It is possible that the mouthpart extrusions are the most important component of the display because a chemical stimulus in the male's saliva is secreted, at least in *N. vitripennis* (van den Assem et al, 1994). It is thought that the mandibular glands are a source of the chemical stimuli (van den Assem, 1986). In a study preformed using males that had their mouths sealed, the males courted normally but never induced receptivity in the female. This therefore suggests that a pheromone in the male's saliva stimulates the female (van den Assem et al, 1994). In fact, the female does not even have to be courted at all to become receptive in certain conditions. When the female I confined in a small space was exposed to a high concentration of the mouthpart pheromone, she spontaneously adopts the copulation posture of raising her abdomen and exposing their genitalia (van den Assem et al, 1994).

Experiments with dummy wasps have demonstrated the possible importance that chemical stimuli have for courtship. *N. vitripennis* males mounted and courted small pieces of plywood and dead females that had female pheromones applied to them. Males would not mount females that were treated with a solvent that apparently covered up the "attractive" scent (van den Assem, 1986).

Pheromones cannot be used for mate finding over long distances. The sex pheromone is thought to only stimulate courtship behavior in wasps that are in close range of each other, about 0-5 mm apart (Ruther, J. et al, 2000). Further studies analyzing how important or necessary the pheromones are to induce receptivity are needed.

It seems no surprise that in *N. vitripennis*, the male would mount whomever he contacted first. Only male genotypes that continue to exist pass genes along, so it makes sense that males would try to mate with the first female contacted, therefore a male can encounter other females and pass along his genes through them as well. Most of the time he was able to copulate with the virgin on the first try. Virgin females almost always open their genital orifice once they have been mounted and courted (King, 2000). Virgin females seem to be receptive regardless of their age and would mate with virgin males and mated males. In a study with *S. cameroni*, virgin females had even mated with a males that copulated 52 times (King, 2000). Their eagerness to mate the first male that mounts them may be evolutionarily adaptive. Since these wasps are only two to three millimeters long and live in such a large place relative to their body size, it may be beneficial to mate the first male that comes along because the odds of finding other males may be low.

The *N. vitripennis* males only copulated with the mated female less than half of the time. Mating more than once is not particularly beneficial for the female, so she has no need to copulate with him. The males maximize the rate of copulations to pass along their genes, therefore they must not give up too soon on these mated females as to not miss an opportunity to inseminate, even if it is not a full batch of sperm. These males should not waste too much time or energy either with these females because they could better use their time and energy courting other females (van den Assem, 1986).

To humans it may make more sense for the male to seek out a virgin rather than the mated female, but perhaps the males chose the mated female because they are more active and the activity caught their attention. As seen in this study and others, mated females were more active than virgin females around five minutes after mating and up to at least two hours after mating (King et al, 2000). Perhaps it is evolutionarily adaptive to choose a more active female. This may demonstrate to the male that the female is healthier and therefore could sire his offspring more successfully and increase the chances that his offspring would have greater fitness.

Factors that may have contributed to results

Relative humidity was observed to have a role in the length of mating, if they did at all. The 10 that did not copulate were in the laboratory when the relative humidity was considerably lower than at other times. It also took longer for the wasps to copulate when the humidity was lower. Perhaps physiologically, the wasps can not function as well in low relative humidity. Maybe wasps thrive in high humidity; after all, most insects tend to be found in humid areas.

As seen in the results, the overall time for contacting and mounting the virgin was less than the time for the mated. Perhaps the male just came across the virgin first or was having trouble seeking out the mated female because she is more active. However, copulation occurred faster with the mated females rather than the virgins. This is probably because when males tried to copulate with mated females first, they were unable to induce receptivity. Therefore, they then had to seek out the virgin, mount, court, and then copulate with her, making their overall time much longer for mating with virgins.

Many more studies need to be preformed to gain greater understanding of the role of the pheromones, leg tapping, and humidity. The importance of why *N. vitripennis* males will mate with already mated females and even choose them over virgins needs to be sought out to further apply these findings to other evolutionary systems of mating.

Table 1A Premounting behavior

	Yes	No	Unclear
Male fan wings?	17	2	1
Male sway side to side?	0	20	0
Female antennae tucked in?	1	15	4

Table 1B Postmounting, precopulatory behavior

	Yes	No	Unclear
Dorsal, posterior mount?	20	0	0
Female antennae extend during courtship?	17	3	0
Female ovipositor pop out?	16	0	4
Female antennae tap?	0	20	0
Male vibrate whole body?	20	0	0
Male's head above female's thorax?	20	0	0
Male's antennae on either side of her?	20	0	0
Male's antennae touch her antennae?	0	20	0
Male's antennae touch her abdomen?	13	1	6
Male's forelegs on thorax?	14	2	4
Male's mouth open?	0	0	20
Male tap his middle legs?	19	0	1
Tap legs in sych. with body vibration?	2	16	2
Male back and side to copulate?	20	0	0
Wings doing anything except shake with body?	0	20	0

Table 1C Postcopulatory behaviors

	Yes	No	Unclear
Male groom immediately after copulation?	0	20	0
Female's antennae still extended?	20	0	0
Male return to same pre-cop. position?	20	0	0
Male back off?	20	0	0
Male abdominal mark tube?	0	2	18
Cont. leg tap after dismount?	0	19	1
Leg tap as dismount?	14	5	1

Table 1D Reencounter behaviors

	Yes	No	Unclear
Does female open genital orifice during post-cop. courtship?	1	5	14
Female walking when male reencounters her?	12	19	0

Table 2A Frequency of whom male chooses first

	Contact	Mount	Copulate
Virgin	61	62	91
Mated	63	63	24

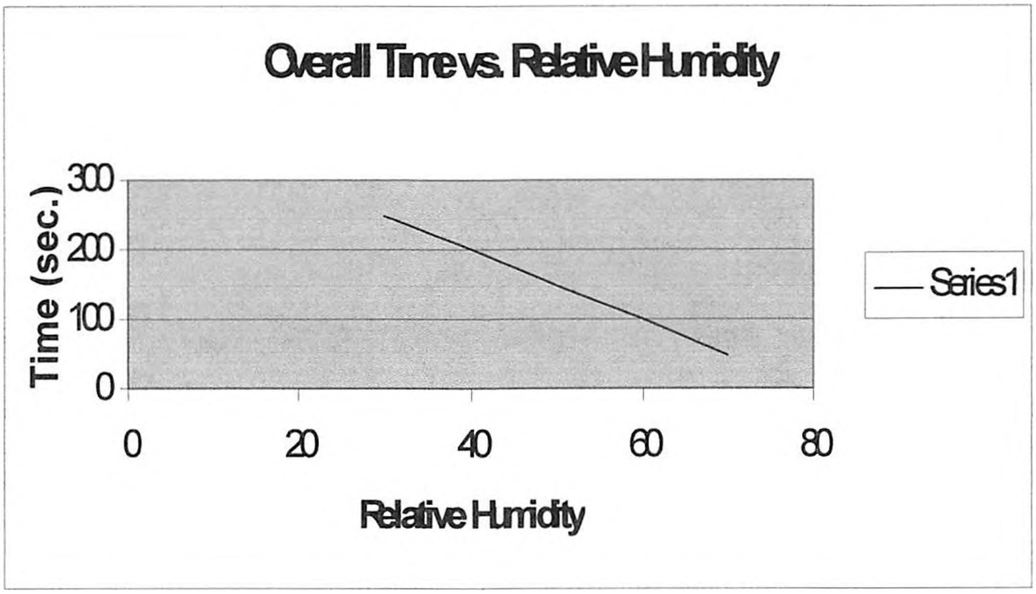
Table 2B Crosstabulation frequency of contact vs. mount

	Mount virgin	Mount mated
Contact virgin	57	4
Contact mated	5	58

Table 2C Crosstabulation frequency of mount vs. copulate

	Copulate virgin	Copulate mated
Mount virgin	61	1
Mount mated	30	23

Figure 1



Overall Time vs. Relative Humidity
Time (sec.)
300
200
100
0
0
20
40
60
80
Relative Humidity
Series1

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