ENVIRONMENTAL FACTORS AFFECTING DISPERSAL BEHAVIOR IN NASONIA VITRIPENNIS (HYM., PTEROMALIDAE)

A Thesis

Presented to

The Faculty of the Department of Biology

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

by

Robert Perry Steele

1985

APPROVAL SHEET

This thesis is submitted in partial fulfillment of
the requirements for the degree of

Master of Arts

Author

Approved, May 1985

Bruce S. Grant

Norman J. Fashing

G. R. Brooks, Jr.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Environmental factors which may influence the dispersal rate of the parasitoid wasp _Nasonia vitripennis_ were examined. Results suggest that male dispersal rate is increased by crowding, decreased by the presence of the host, and not affected by the presence of caged females. Female dispersal rate was increased by crowding and, in the case of mated females, apparently also by the presence of other mating wasps. Mated females tended to disperse faster than virgin females in both in the presence and absence of other mating wasps.

ENVIRONMENTAL FACTORS AFFECTING DISPERSAL BEHAVIOR IN *NASONIA VITRIPENNIS* (HYM., PTEROMALIDAE)

INTRODUCTION

This project was designed to investigate the environmental factors which influence the dispersal behavior of the parasitoid wasp *Nasonia vitripennis*. In nature, *N. vitripennis* females oviposit on the pupae of a variety of carrion flies (Whiting, 1967). The wasp eggs hatch and go through larval and pupal stages within the puparium of their host, using the host for food. Male *N. vitripennis* ordinarily eclose somewhat sooner than females, but do not mate until emergence from the host puparium. Females have fully developed wings and can fly, whereas males have much smaller wings and cannot fly. Males remain in the vicinity of the host following emergence and mate with the females as they emerge (Whiting, 1967; King, et al., 1969; Van den Assem, et al., 1981). According to King et al. (1969): 1) males are strongly attracted to hosts and prefer hosts that have produced parasites to those that are about to; 2) males prefer parasitized hosts to unparasitized hosts; and 3) virgin males have a higher level of dispersal activity than mated males. Females ordinarily mate only once, although double mating occasionally occurs, apparently when a second male copulates with a still-receptive female immediately following the completion of mating by the first male (Holmes, 1974).

Van den Assem et al. (1981) state that during courtship males discharge a pheromone which is necessary for the initiation of sexual receptivity in virgin females. This pheromone is airborne (Van den Assem, et al., 1981) and may be extruded from the mandibular glands (Van den Assem, et al., 1980a).

After mating, females disperse by walking and flying, eventually locating a fresh host. Virgin females may also disperse and lay unfertilized eggs, all of which develop into males.

Males repeatedly rub the tip of their abdomen on the substrate after mating. Barrass (1969) called this behavior "abdomen dipping", and described it in the following words: "the most posterior segments of the abdomen move almost imperceptively upwards and then the abdomen as a whole is flexed ventrally between the metacentric limbs, a drop of fluid appears at the tip of the abdomen as it touches the substratum and then the male walks forwards depositing the fluid as a streak." According to Barrass, males abdomen dipped more frequently after courting receptive females than after courting nonreceptive females. Abdomen dipping also occasionally occurs in males that have not yet been in the presence of a female (Barrass, 1969).

Van den Assem et al. (1980a) suggest that abdomen dipping behavior results in the deposition of a pheromene which males use to mark the spot where they mated. Their experiments indicate that: 1) mated males tend to remain in the vicinity of where they mated (and presumably subsequently abdomen dipped), 2) males which did not mate and abdomen dip in a particular location will nonetheless remain near that location if mating by other wasps had occurred there previously (Van den Assem, et al., 1980a), 3) the first male to mate in a particular area will chase other males from that area (Van den Assem, et al., 1980b, cited in Van den Assem, et al., 1980a). King et al. (1969) have observed such territorial behavior of male *N. vitripennis* around parasitized hosts.

The presumed advantage to a male of marking and holding a territory is that, since the location of the territory is the site of a previous mating and most matings occur near the host, it is likely that staying near the site of a previous mating will improve the male's chances of encountering another female over the probability of encountering another female in a randomly chosen territory or during a random search (King et al., 1969; Van den Assem, et al., 1980a).

Nagel and Pimentel (1963) have shown that: (1) female dispersal rate is retarded by increasing host density, and (2) females disperse faster from parasitized hosts than from unparasitized hosts. It has been stated that females do not begin dispersing from a chamber containing hosts until all of the hosts have been parasitized (Chabora and Pimentel, 1970); however, Grant (unpubl.) has data indicating that females do sometimes leave dispersal chambers containing unparasitezed hosts.

The types of environmental factors that would be expected to influence male N. vitripennis dispersal include: 1) presence of different numbers of males (crowding), 2) the presence or absence of females, 3) the previous presence of mating, and 4) the presence or absence of hosts near the host from which the male eclosed, and the conditon (parasitized or unparasitized) of those nearby hosts. Environmental factors expected to influence female dispersal behavior are: 1) presence of other females (crowding), and 2) presence or absence of mating by other wasps. The effect of hosts on female dispersal has been investigated in a previous study by Grant (unpubl.).

In this project, tendency of both males and females to disperse under varying conditions of crowding was measured separately with I-mazes. The effects of the presence of 1) caged females, 2) substrates upon which mating had occurred, and 3) hosts on male dispersal were evaluated using I-mazes and observation arenas. The influence of the presence of other caged mating wasps on both mated and virgin female dispersal rate was measured with I-mazes.

MATERIALS AND METHODS

Male Dispersal

1. The effect of crowding on male dispersal was investigated using a simple I-maze made from six one ounce polyethylene funneled dropper bottles placed end-to-end (modified from Grant and Mettler, 1969) (see Fig 1). Dispersal runs were made with the maze hanging vertically. Runs were begun by releasing either five or twenty males from a gelatin capsule into the lowermost bottle of each maze. Runs were ended by inverting the maze. When the maze was inverted, a BB (.18 inch diameter steel ball) previously placed in each bottle fell into the funneled neck of the bottle, thereby preventing wasps from passing through the funnel into the next bottle. Thus all wasps were trapped in the bottle in which they were located when the maze was inverted. Scoring was done by counting the number of wasps in each bottle after inversion of the maze.

Data taken provided: 1) the number of wasps that remained in the bottom bottle at the end of the run, expressed as a proportion (P1) of all wasps put in the maze, and 2) mean score (symbolized as "Y"). Mean score was calculated by assigning the bottom bottle in a maze one point, the second bottle two points, and so forth. The number of wasps in each bottle was multiplied by the point value of that bottle. The sum of the point totals of each bottle in a maze was divided by the number of wasps dispersing in that maze to give a mean dispersal score for the trial.

2. The effect on male dispersal of the presence or absence of females was investigated using an I-maze consisting of two bottles. Virgin females were placed in small "cages" made from No. 1 gelatin pill capsules punctured with twelve holes too small to permit the passage of wasps. A cage containing females was placed in the bottom bottle of each maze in the experimental runs. The control runs were done using an empty cage (perforated capsule).

The effect of the presence or absence of females on horizontal distribiton of males was also measured. In this design a nine cm diameter plastic petri dish lid was inverted over a sheet of white paper on which a straight line was drawn. The lid was oriented so that when viewed from above it was bisected by the line on the paper. One half of the arena contained two perforated gelatin capsule cages with one virgin female in each. Two empty perforated capsules were placed in the other half of the arena as a control. The runs were begun by releasing ten males along the axis of the line dividing the arena. Wasps in each half of the arena were counted at one minute intervals. A mean number of wasps on each side was calculated by dividing the sum of the counts on each side by the total number of counts. This value was converted to a proportion by dividing by the sum of the means from each side.

3. The previous presence of mating on male dispersal was evaluated to test Van den Assem et al.'s (1980a) substrate marking hypothesis. This experiment was done using two-bottle I-mazes. The bottom bottle in the experimental runs contained two halves of a gelatin capsule in which mating had occurred immediately prior to beginning of the run. The bottom bottle of the control runs contained two untreated gelatin capsule halves.

4. The effect of host condtion on male dispersal was measured using both the two-bottle I-maze and the petri dish designs. The hosts (pupae of the flesh fly, *Sarcophaga bullata*) were prepared in three ways: 1) parasitezed host pupae from stocks from which male and female *N. vitripennis* were expected to begin emerging on the day following the run (henceforth called "hit"), 2) pupae which had not previously been exposed to wasps ("unhit"), and 3) parasitized pupae from stocks which had within the two days prior to the run produced both male and female wasps. Pupae for the third treatment were cut in half radially and emptied of any remaining wasps and unconsumed host. This condition will henceforth be called "spent halves". In experimental runs for hit and unhit hosts the effect on male dispersal of two host pupae was compared to the effect of two empty gelatin capsules of roughly the same

size as the host. In experiments using spent halves, four host halves were used as the experimental treatment and four gelatin capsule halves were used as a control. The possibility that any effect seen with the above design was due to attraction or repulsion of the wasps to or from the gelatin capsules in the control runs was tested by doing petri dish runs with two empty capsules on one side of a petri dish arena and no capsules on the other side. There was no significant difference ($p= 0.4062$ by the Mann-Whitney U test) between sides in seven runs of this experiment (see Table 11). Thus the presence of gelatin capsules does not appear to bias the results of the host experiments.

Female Dispersal

The dispersal rates of virgin and mated females were compared under the following conditions: 1) presence of other females (crowding), and 2) presence or absence of mating by other wasps. Crowding was examined by comparing the dispersal rate of five females in a two-bottle maze with that of twenty females in identical mazes. The effect of crowding on mated and virgin females was assessed separately.

The effects of the presence or absence of other mating wasps on the dispersal rates of mated and virgin females was investigated by running mated and virgin females separately in ten-bottle I-mazes. The bottom bottle of each experimental run held a cage containing ten males and ten females that had been previously stored separately as virgins. Active mating began when the sexes were united in cages immediately prior to the beginning of the dispersal run. The control mazes held empty cages in the bottom bottle. Runs were begun by releasing either ten mated or virgin females into the bottom bottle of each maze. Thus the four different treatments in this experiment were: 1) virgin females dispersing from bottles containing cages in which mating was occurring (henceforth called "mating bottles"). In tables this treatment will be called "m/vF"); 2) mating bottles from which mated females are dispersing (to be labeled "m/mF"); 3) virgin females dispersing from bottles containing empty cages ("nonmating bottles"); (this treatment is labeled "nm/vF"); 4) nonmating bottles from which mated females are dispersing (labeled "nm/mF").

A note should be made at this point about the differences between the I-maze and petri dish designs. The I-maze measures rate of vetrical dispersal inasmuch as it would be expected that a wasp traveling relatively rapidly would find its way through more bottles than one moving at a slower rate. Because the funneled neck of an individual dispersal bottle narrows to a tip that protrudes above the inside bottom of the next bottle, the dispersal of an individual wasp is assumed to be only from a lower bottle to the next highest one and not the reverse. This assumption was tested by placing 10 wasps in each of the upper bottles of six two-bottle I-mazes. After six hours, only six of the fifty-five wasps recovered had moved to the lower bottle. This represents a rate of movement much lower than that of all other experiments done with I-mazes presented here. Thus for the purposes of these experiments dispersal measured in I-mazes is essentially unidirectional: bottom to top. In the petri dish design there is no physical aspect of the arena to impede the passage of a wasp from one side to the other. Scored over time, the petri dish design therefore measures the tendency of a wasp to remain on one side of the arena, while the I-maze measures the tendency of a wasp to leave the bottle in which it is located.

The wasps used in these experiments were of the Fresh Pond strain, a lab stock started from a single wild female. Experimental wasps were raised in an incubator on lab-produced pupae of the flesh fly *Sarcophaga bullata*. Runs were done at temperatures ranging from 25-30 C, with the majority at 25.6 C. Wasps of identical age were run in each treatment on a given day for every experiment. Roughly equal numbers of replicates for each treatment in every experiment were run every time each experiment was done. All statistical computations used in this paper, unless otherwise designated, are described in Sokal and Rohlf, 1981.

RESULTS

Male Dispersal: Social Effects

1. For the male crowding dispersal experiments the statistics compared were: 1) proportion of wasps remaining in the first bottle of the six-bottle I-maze at the end of the run ("P1"), and 2) mean dispersal of wasps ("Y") (see Table 1). Both of these measures show a significantly greater dispersal rate in each experimetal run by the Mann-Whitney U test for males in more crowded starting conditions (30 males dispersing) than for males in less crowded conditions (5 males).

2. The results from the experiments examining male dispersal from the presence of caged virgin females were inconclusive (see Tables 2 & 3). Although four of the five sets of I-maze runs had fewer males dispersing from bottles containing females than from control bottles, only one of these differences came close to significance (run 1 in Table 2; p=0.0734 by the Mann-Whitney U test).

The proportions of males on either side of the arena in the petri dish experiment (see Table 3) were not significantly different (p=0.3367) when compared by the Mann-Whitney U test; however, the skew in the distribution of the raw data is on the side of the dish with cages not containing females. This result suggests that distribution of males in this experiment is random with respect to the treatment.

From the results of the petri dish and I-maze experiments it seems evident that if there is an effect by caged virgin females on male dispersal behavior, it is not shown with these designs.

3. The trend of the two-bottle I-maze previous presence of mating experiments on male dispersal was that males always dispersed more slowly from bottles containing opened gelatin capsules in which mating had occurred (see Table 4). In one of these runs the difference was highly significant (run 3), and in another almost significant (run 2). These results suggest that male dispersal rate is decreased by the previous presence of mating on a substrate (the gelatin capsule) to which the dispersing males have access. This finding is consistent with that of Van den Assem et al. (1980a). substrate marking hypotheses described above.

Male Dispersal: Host Effects

Both two-bottle I-maze and petri dish designs were used in the experiments on the attractiveness of host pupae to male *N. vitripennis*. In both designs, the effects on male dispersal of three types of host condition were compared to the effect of empty gelatin capsule controls. The host conditions were: (1) hit, (2) unhit, (3) spent host halves.

For the petri dish design, host pupae attracted significantly more males than did control capsules (see Table 5). The results of the two-bottle I-maze host-effect experiments were less clear (see Table 6).

Males appear to be attracted more strongly to cleaned-out hosts that had produced wasps than to control capsules. This conclusion is supported by direct observations of males climbing into and on the host halves during petri dish runs. The number of males doing this to one host half was frequently sufficient to move the host half a substantial distance from its original position. No such behavior of the males was observed with respect to the gelatin capsule in the other half of the arena. Out of all the runs made, only once was a male observed to enter one of the control gelatin capsules.

Female Dispersal

1. For the female crowding experiments, two pilots runs of respectively three and two replicates of mazes using 5 and 20 mated females showed higher mean dispersal rates for the more crowded mazes (20 females) than for the less crowded (5 females) mazes. In a run of this experiment with ten replicates each of both conditons, dispersal rate was significantly faster for the more crowded mated females (see Table 7).

When the same design as above was run with virgin females, the results of the first run had extreme variance within treatments and were discarded. In a second run, virgin females dispersed faster when crowded as compared to less crowded conditions at a probability level very close to significance (see Table 7).

Thus both mated and virgin females appear to disperse at a higher rate from crowded conditions. It would be interesting to do more runs in order to see if crowding affects the dispersal rates of virgin and mated females differently.

2. The results of the ten-bottle I-maze female dispersal experiments on presence of mating are summarized in Tables 8 & 9. Both mean dispersal (Y) and the proportion of wasps in the first bottle at the end of the run (P1) are reported.

In two way analysis of variance using P1, there were significant differences both between treatments ($p < .001$) and between different runs of the same treatments ($p < .0001$). For the same test run using Y, there were similar significant differences ($p = .001$ and $p < .0001$, respectively).

Thus there are highly significant differences between treatments of this experiment. Paired T-tests were used to compare the results of individual treatments (see Table 10). Because four comparisons were made, the "experimentwise error rate" (Bonferroni's correction) required adjustment of the significance level from alpha = 0.05 to alpha prime = 0.0127 (see Sokal and Rohlf, 1981). Inspection of Table 10 shows that although only one of the comparisons made is siginificant by alpha prime, several of the others approach significance.

Beginning with the first comparison (m/vF vs. nm/vF), the dispersal rate of virgin females does not seem to be affected by the presence of other wasps mating inside perforated capsules. However, the presence of mating appears to significantly increase the dispersal rate of mated females (comparison 2, m/mF vs. nm/mF). When mated and virgin females are compared under mating conditions (comparison 3, m/vF vs. m/mF), the comparison by P1 approaches significance, with mated females dispersing at a greater rate than virgin females. A difference in Y approaches significance when mated and virgin females are compared in the absence of other mating wasps (comparison 4, nm/vF vs. nm/mF), with mated females dispersing at a greater rate.

The difference in relative dispersal rates of the four conditions (see Table 10) is difficult to explain. P1 was intended as a measure of the tendency of wasps to leave or remain in the first bottle of the maze, and thus is a measure of the attractiveness of any treatment (such as caged mating wasps) done on that bottle. Because Y reflects the average dispersal of wasps throughout the entire maze, it is meant to reflect both treatment effects and the intrinsic dispersal tendency of a wasp independent of treatment. Therefore P1 seems a more appropriate comparison of groups run in the presence of other mating wasps, and Y a better measure of nonmating runs.

These trends can be summaraized as: 1) mated females dispersed faster in the presence of mating, 2) virgin females were not affected by the presence of mating, and 3) mated females dispersed faster than virgins regardless of the local mating conditions.

In all of the male and female dispersal experiments reported above, the variance between different runs of the same treatment done on different days was so great that it is not appropriate to pool data from different days. Variance between replicates done on the same day was also large. Suspected sources of this variance include: 1) Age of the dispersing wasps. Age was tightly controlled by day, but not by hour. If age in hours makes a difference in *N. vitripennis* dispersal behavior, then this could be a source of variance. Increasing age has been shown to increase dispersal rate in *Drosophila pseodoobscura* (Dobzhanksy and Powell, 1974; Crumpacker and Williams, 1973). In *N. vitripennis*, age appears to influence mating behavior (Barrass, 1960). 2) Size effect. Wasps used were not controlled for size. It is not implausible that size could be associated with level of activity and hence dispersal rate. 3) Stock host effects. Mixing of wasps run to insure equal distribution of wasps from each host to different treatments was not done. Thus if wasps obtained from different hosts have different levels of dispersal activity due to nutrition or other host effects, then some of the observed variance is probably due to this factor. 4) Temperature. Dobzhansky and Wright (1943) have reported a significant influence of temperature on *Drosophila pseudoobscura* dispersal.

Temperature was controlled in the experiments reported in this paper and thus is not likely to be to be a significant factor in the variance of the results. 5) Relative Humidity. As relative humidity varied widely from day to day, it may well contribute substantially to the variance. 6) Time of day when runs were performed. This factor was not controlled, and thus may contribute to the variance.

DISCUSSION

Unpublished experiments by Grant (personal communication) suggest that males remain near host pupae that are actively producing females until these pupae are spent, at which time the males begin to disperse. This result leads to the question of what are the factors that allow a male to orient himself to the host so that he will be able to mate with emerging females. The crowding experiments reported above suggest that males disperse away from one another when there are no other environmental factors (hosts, females) to cause them to congregate. In the absence of attractive factors, increase in dispersal rate under crowding may simply be due to an increased activity level of crowded males caused by a greater interaction rate due to closer proximity. An exaggeration of normal dispersal rates in *Drosophila pseudoobscura* due to crowding has been suggested by Dobzhansky and Powell (1974) and Wallace (1966). It would be of interest to do experiments to determine whether the male crowding effect operates under conditions where there are environmental factors present that attract males. Experiments by Grant (unpubl.) indicate that when hosts are available, female *N. vitripennis* dispersal rate is not increased by crowding.

Females inside of gelatin capsules do not seem to attract males. This could be because female pheromones are absorbed by the gelatin capsule, but when it is considered that males will court almost any object roughly the size of a female wasp, including other males (Whiting, 1967), the more likely explanation seems to be that males do not receive their initial information about the sex of another wasp from generally broadcast, airborne pheromones, but rather from behaviors of the wasps they court (perhaps including pheromones released in response to courtship, or antennal or limb movements). This hypothesis would predict that males would not remain in the vicinity of hosts because they detected females inside about to eclose, but because the males were able to identify the host as such and remain near the host because it is the only object in the environment likely to produce females.

This hypothesis is consistent with the results of the male dispersal host experiments reported above, but seems inconsistent with the findings of King et al (1969) that males can distingish between hit and unhit hosts. The results of King et al suggest that males detect developing females within the host and prefer hit hosts for that reason. Thus it appears that males do not detect females in cages which permit the free passage of air, but can detect females within a hit host.

A possible explanation for this result is that parasitization of a host pupa might change its odor. This hypothetical difference might be detected by male N. vitripennis. The fact reported above that males are attracted to spent halves of pupae suggests that it is some factor other than the females themselves that attracts males. It would be interesting to see if hosts that will produce only males are any less attractive to males than hosts that will produce both males and females -- if so this would imply that males are able to assess the sex of wasps within unopened pupae.

The general conclusions that the results of the male dispersal experiments suggest are : 1) males orient primarily to the host; 2) males tend to remain in the vicinity of sites where previous matings by other wasps have occurred; thus the tencency to orient to the host would be magnified when newly emerged females mate with the males congregated outside the host; and 3) crowding increases the dispersal rate of males. Further experiments will be necessary to determine whether the effect of crowding is antagonistic to the tendency of males to remain near the host and near sites of previous matings.

The results of the female dispersal experiments suggest that: 1) mated and virgin females disperse at a higher rate under crowded conditions; 2) virgin females disperse at a constant rate regardless of the presence of absence of mating by other wasps; and 3) the dispersal rate of a female increases after mating, and may be further increased by the presence of mating by other wasps.

If it were the case that virgin females were not likely to be mated unless they remained near the host for some period of time following emergence from the host, then it would be expected that virgin females might be attracted to the presence of mating. It is, however, not likely that many females would remain unmated after passing through the group of males that aggregate outside the host as females are eclosing (Grant, unpubl. data). If females do not ordinarily encounter situations where they would need to seek males, then it is not suprising that the dispersal rate of virgin females is not influenced by the presence of mating by other wasps. This suggests that virgin females may facilitate mating simply by dispersing more slowly without actively seeking out males. This hypothesis would predict that virgin females may be less influenced by crowding than mated females; such a prediction would be simple to test using the design described above for crowding experiments. An increased responsiveness to crowding may be the proximate mechanism that causes mated females to disperse at a higher rate than virgins.

After a female is mated, it would be important for her to leave the site of mating rather than remaining to be courted by additional males. Remaining would represent time lost to search for new hosts. Furthermore, there are no obvious competitive reasons for females to stay around males after mating against the possibility of later mating with a more fit male because sperm displacement virtually does not occur in _N. vitripennis_ (Holmes, 1974). Thus it is plausible that mated females would disperse at a faster rate from the presence of other mating wasps. Having mated therefore appears to enhance a female's already high rate of dispersal.

In summary, male and female dispersal behavior appear to promote conditons that would be expected to facilitate mating by both sexes. Further work in the areas described above should be done in order to test this conclusion.

TABLE 1

Effect of Crowding on Male Dispersal in Six-Bottle I-Mazes

run	5 males			30 males			significance		run
no.	Y	Pl	a	Y	Pl	a	by Pl	by Y	length
1	1.9	.400	2	4.41	.207	1	-	-	2 hr.
2	1.0	1.00	1	2.17	.233	1	-	-	1 hr.
3	1.25	.850	4	3.03	.181	3	.0323	.0323	1 hr.
4	1.29	.743	7	2.02	.385	7	.0330	.0175	1 hr.
5	1.3	.700	4	2.21	.180	4	.0209	.0209	26 min.

Pl = proportion of wasps in the first bottle; a = number of mazes per treatment. The Mann-Whitney U test was used to test for differences between the treatments.

TABLE 2

Effect of Caged Virgin Females on Male Dispersal in Two Bottle I-Mazes

Run No.	P1 w/ females,	a	P1 w/o females,	a	No. females per perf. cap.	Run length
1	.9583	15	.80952	15	not recorded	3.5 hr.
2	.5677	31	.4937	32	not recorded	13 hr.
3	.8889	27	.8414	29	1	3.5 hr.
4	.800	25	.837	27	3	5 hr.
5	.600	40	.575	40	1	4 hr.

Note: In runs 1-4 five males were run per maze. In run 5 one male was run per maze. P1 = proportion of wasps remaining in the first bottle at the end of the run; a = number of mazes per treatment. The Mann-Whitney U test was used to test for differences between treatments.

TABLE 3

Effect of Caged Virgin Females on Male Distirbution in Petri Dish Arenas

Dish no.	1	2	3	4	5	6
Sum w/ females	61	64	83	32	61	37
Sum w/o females	79	76	67	58	87	23
No. of readings	15	15	15	9	15	6

Note: The Mann-Whitney U test done on this data compared the proportion of wasps on each side of the arena.

TABLE 4

Effect of the Previous Presence of Mating on Male Dispersal in Two-Bottle I-Mazes

Run No.	Pl treated	a	Pl untreated,	a	sig. by arcsin test	Run length
1	.9226	(31)	.8839	(31)	.326	2.2 hr.
2	.600	(40)	.395	(38)	.0688	3.5 hr.
3	.8958	(43)	.5000	(42)	.000064	3.5 hr.
4	.8571	(42)	.7381	(42)	.1706	3.5 hr.

Note: Run 1 had 5 males dispersing per maze. Runs 2-4 had one male dispersing per maze. The arcsin test is a test for the diffenceces between two proportions (Sokal and Rohlf, 1969). Pl = proportion of wasps remaining in the first bottle at the end of the run; a = number of mazes per treatment.

TABLE 5

Effect of Hosts on Male Distribution in Petri Dish Arenas

		Sum on the side of the arena			
Treatment	Arena No.	w/host	a	w/o host	a
Halves	1	149	20	51	20
	2	162	20	38	20
	3	144	20	56	20
	4	170	31	140	31
	5	223	31	87	31
	6	239	31	71	31
Unhit	1	149	26	59	26
	2	180	26	80	26
	3	171	26	89	26
	4	152	26	108	26
	5	169	26	91	26
	6	146	26	114	26
	7	168	26	92	26
Hit	1	189	26	71	26
	2	183	26	77	26
	3	188	26	72	26
	4	192	26	68	26
	5	171	26	63	26
	6	212	26	48	26

a = number of readings per maze

Significance by the Mann-Whitney U test:
Treatment - Halves, $p = 0.0039$
- Unhit, $p = 0.0017$
- Hit, $p = 0.0039$

TABLE 6

Effect of Hosts on Male Dispersal in Two-Bottle I-Mazes

Treatment	Run No.	P1 w/host	a	P1 w/o host	a
spent halves	1	.900	6	.700	6
spent halves	2	.680	10	.710	10
hit	1	.6857	7	.700	8
unhit	1	.7143	7	.500	8

Note: P1 = proportion of wasps remaining in the first bottle at the end of the run; a = number of mazes per treatment. All differences between treatments were nonsignificant by the Mann-Whitney U test.

TABLE 7

Effect of Crowding on Female Dispersal in Two-Bottle I-Mazes

I. Mated Females

Maze No.		1	2	3	4	5	6	7	8	9	10
number in bottom bottle	5 Females	4	4	4	5	5	4	5	4	3	5
	20 Females	8	9	6	11	7	7	15	12	10	13

Significance by Mann-Whitney U Test: $p = 0.0003$

II. Virgin Females

Maze No.		1	2	3	4
number in bottom bottle	5 Females	5	4	4	5
	20 Females	16	12	11	

Significance by Mann-Whitney U Test: $p = 0.0640$

TABLE 8

Effect of the Presence of Mating by Other Wasps on Female Dispersal in Ten-Bottle I-Mazes -- Scored by Y

	m/vF			m/mF			nm/vF			nm/mF		
Run No.	Y	SD	a	Y	SD	a	Y	SD	a	Y	SD	a
1	1.60	.283	2	3.50	.693	3	2.23	.299	4	2.15	.354	2
2	6.50	.141	2	5.20	.141	2	3.90	.000	1	3.95	1.061	2
3	2.43	.603	3	3.13	1.737	4	2.63	.519	4	2.55	.350	2
4	3.50	1.697	2	3.20	.000	1	2.85	1.344	2	2.95	1.344	2
5	3.20	.566	2	4.95	.311	4	2.27	.666	3	3.57	2.013	3
6	2.10	.392	4	2.28	.877	4	1.70	.141	2	1.98	.222	4
7	1.30	.265	3	2.40	.700	3	1.10	.082	4	2.10	.755	3
mean	2.73			3.62			2.18			2.67		
sum			18			21			20			18

SD = standard deviation; a = number of mazes per treatment

TABLE 9

Effect of Presence of Mating by Other Wasps on Female Dispersal in Ten-Bottle I-Mazes -- Scored by Pl

	m/vF			m/mF			nm/vF			nm/mF		
Run No.	Pl	SD	a	Pl	SD	a	Pl	SD	a	Pl	SD	a
1	.650	.071	2	.400	.173	3	.375	.096	4	.550	.071	2
2	.050	.071	2	.100	.141	2	.100	.000	1	.250	.212	2
3	.333	.153	3	.175	.096	4	.400	.141	4	.200	.000	2
4	.200	.283	2	.200	.000	1	.300	.141	2	.200	.283	2
5	.350	.071	2	.125	.126	4	.467	.115	3	.333	.115	3
6	.525	.171	4	.400	.294	4	.550	.071	2	.425	.096	4
7	.900	.100	3	.333	.252	3	.850	.129	4	.667	.252	3
mean	.461			.257			.485			.394		
sum			18			21			20			18

SD = standard deviation; a = number of mazes per treatment

TABLE 10

Comparison of the Effect of Mating and the Presence of Other Mating Wasps on Female Dispersal with Paired T-Tests

Comparison	by Pl: t-value	p	by Y: t-value	p
(1) m/vF vs. nm/vF	0.10	.927	1.45	.198
(2) m/mF vs. nm/mF	2.79	.031	3.85	.008
(3) m/vF vs. m/mF	2.38	.055	1.33	.233
(4) nm/vF vs. nm/mF	1.01	.350	1.76	.130

Note that for a significance level of alpha = 0.05, the adjusted value of alpha by Bonferroni's correction = 0.0127 (see text).

m/mF = maze containing caged mating wasps in which uncaged mated females were dispersing; m/vF = maze containing caged mating wasps in which uncaged virgin females were dispersing; nm/mF = mazes containing empty cages in which mated females were dispersing; nm/vF = mazes containing empty cages in which virgin females were dispersing

Comparison of mean dispersal rates:
for Y: m/mF > m/vF = nm/mF > nm/vF
for Pl: m/mF > nm/mF > m/vF > nm/vF

TABLE 11

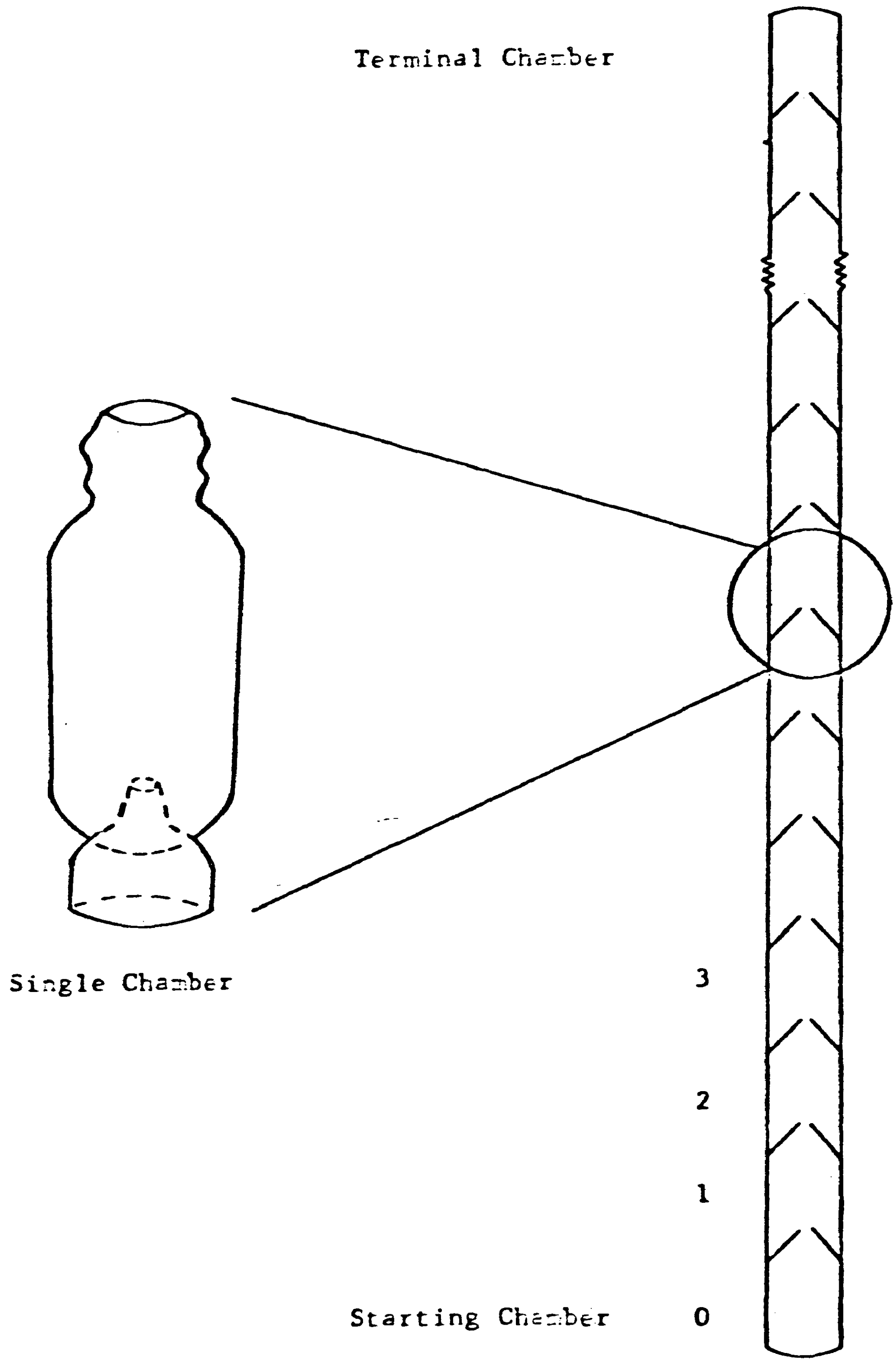
Effect of Empty Unopened Gelatin Capsules on Distribution of Males in Petri Dish Arenas

	Sum on the Side of the Arena	
Arena No.	w/capsules	w/o capsules
1	84	176
2	158	102
3	115	145
4	119	106
5	129	81
6	121	113
7	133	101

Note: The Mann-Whitney U Test done on this data compared the _proportion_ of wasps on each side of the arena.

FIGURE 1

Diagram of I-maze constructed from a linear sequence of one ounce polyethylene dropper bottles.


Terminal Chamber
Single Chamber
3
2
1
Starting Chamber
0

APPENDIX - RAW DATA

All values reported below for I-mazes represent the number of wasps counted in each bottle of the maze.

I. Male Dispersal: Crowding--Six-Bottle I-Maze Design

Note: Raw data for runs 1 & 2 are not reported because the sample sizes were too small for testing differences between treatments.

Run 3

		maze no.						
		1	2	3	4	5	6	7
bottle no.	1	3	2	7	5	5	7	4
	2	1	3	5	0	0	8	1
	3	0	6	8	0	0	9	0
	4	1	11	6	0	0	2	0
	5	0	5	4	0	0	1	0
	6	0	2	0	0	0	2	0
total		5	29	30	5	5	29	5

Run 4

maze no.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
	1	4	4	9	5	3	13	5	4	7	28	4	14	4	2
	2	0	1	12	7	2	12	0	1	8	2	6	14	1	3
bottle	3	1	0	8	16	0	4	0	0	8	0	15	1	0	0
no.	4	0	0	1	1	0	0	0	0	3	0	2	1	0	0
	5	0	0	0	0	0	0	0	0	3	0	1	0	0	0
	6	0	0	0	0	0	0	0	0	1	0	0	0	0	0
total		5	5	30	29	5	29	5	5	30	30	30	30	5	5

Run 5

maze no.

		1	2	3	4	5	6	7	8
	1	3	9	4	11	2	7	5	6
	2	2	10	1	10	3	3	0	21
bottle	3	0	4	0	8	0	12	0	1
no.	4	0	6	0	1	0	8	0	0
	5	0	0	0	0	0	0	0	1
	6	0	0	0	0	0	0	0	0
total		5	29	5	30	5	30	5	29

II. Male Dispersal: Presence of Females -- Two-Bottle I-Maze Design

Note: In ratios reported below, the numerator refers to the number of wasps in the top bottle; the denomenator refers to the number of wasps in the bottom bottle -- thus 2/3 = two wasps in the top bottle and three wasps in the bottom bottle.

Run 1: five males dispersing per maze

w/female	no. observed	w/o female	no. observed
0/5	12	0/5	8
1/4	3	1/4	3
2/3	0	2/3	3
3/2	0	3/2	1
4/1	0	4/1	0
5/0	0	5/0	0

Run 2: five males dispersing per maze

w/female	no. observed	w/o female	no. observed
0/5	7	0/5	5
1/4	5	1/4	4
2/3	4	2/3	5
3/2	7	3/2	6

4/1	5	4/1	11
5/0	1	5/0	1

Run 3: five males dispersing per maze

w/female	no. observed	w/o female	no. observed
0/5	11	0/5	15
1/4	5	1/4	4
2/3	10	2/3	6
3/2	0	3/2	2
4/1	0	4/1	0
5/0	0	5/0	0

Run 4: one male dispersing per maze

w/female	no. observed	w/o female	no. observed
0/1	24	0/1	23
1/0	16	1/0	17

Male Dispersal: Previous Presence of Mating - I-Maze Design

Run 1: five males dispersing per maze

treated	no. observed	untreated	no. observed
0/5	23	0/5	19
1/4	5	1/4	9
2/3	2	2/3	2
3/2	1	3/2	0
4/1	0	4/1	0
5/0	0	5/0	1

Run 2: one male dispersing per maze

treated	no. observed	untreated	no. observed
0/1	24	0/1	15
1/0	16	1/0	23

Run 3: one male dispersing per maze

treated	no. observed	untreated	no. observed
0/1	38	0/1	21
1/0	5	1/0	21

Run 4: one male dispersing per maze

treated	no. observed	untreated	no. observed
0/1	36	0/1	31
1/0	6	1/0	11

Male Dispersal: Host Effect -- Two-Bottle I-Maze Design

Spent Halves

Run 1: five males dispersing per maze

w/host	no. observed	w/o host	no. observed
0/4	1	0/4	0
0/5	3	0/5	0
1/4	2	1/4	3
2/3	0	2/3	0
3/2	0	3/2	0
4/1	0	4/1	0
5/0	0	5/0	0

Run 2: five males dispersing per maze

w/host	no. observed	w/o host	no. observed
0/4	1	0/4	0
0/5	1	0/5	4
1/3	1	1/3	0
1/4	4	1/4	1
2/3	0	2/3	3
3/2	2	3/2	1
4/1	1	4/1	1
5/0	0	5/0	0

Hit

Run 1: five males dispersing per maze

w/host	no. observed	w/o host	no. observed
0/4	1	0/4	0
0/5	3	0/5	0
1/4	2	1/4	3
2/3	0	2/3	3
3/2	0	3/2	0
4/1	0	4/1	0
5/0	0	5/0	0

Unhit

Run 1: five males dispersing per maze

w/host	no. observed	w/o host	no. observed
0/5	3	0/5	2
1/4	0	1/4	1
2/3	2	2/3	1
3/2	2	3/2	0
4/1	0	4/1	3
5/0	0	5/0	1

BIBLIOGRAPHY

Barrass, R., 1960. The effect of age on the performance of an innate behavior pattern in *Mormoniella vitripennis* (Walker) (Hymenoptera, Pteromalidae). Behaviour 15: 210-218.

-----------, 1969. Preening and abdomen dipping by male *Mormoniella vitripennis* (Walker) (Hymenoptera, Pteromalidae) after courtship. Behavior 35: 304-312.

Chabora, P., D. Pimentel, 1970. Patterns of evolution in parasite-host systems. Ann. Ent. Soc. Am. 63: 479-486.

Crumpacker, D., J. Williams, 1973. Density, dispersion, and population structure in *Drosophila pseodoobscura*. Ecological Monographs 43: 499-538.

Dobzhansky, T., J. Powell, 1974. Rates of dispersal of *Drosophila pseudoobscura* and its relatives. Proc. Roy. Soc. Lond. B. 187: 281-298.

Dobzhansky, T., S. Wright, 1943. Genetics of natural populations: x. Dispersion rates in *Drosophila pseudoobscura*. Genetics 28: 304-340.

Grant, B., L. E. Mettler, 1969. Disruptive and stabilizing selection on the "escape" behavior of *Drosophila melanogaster*. Genetics 62: 625-637.

Holmes, H., 1974. Patterns of sperm competition in *Nasonia vitripennis*. Can. J. Genet. Cytol. 16: 789-795.

King, P., R. Askew, C. Sanger, 1969. The detection of parasitized hosts by males of *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) and some possible implications. Proc. R. Ent. Soc. Lond. (A). 4: 85-90.

Nagel, W., D. Pimemtel, 1963. Some ecological attributes of a pteromalid parasitic wasp and its housefly host. Canadian Entomologist 95: 208-213.

Sokal, R., F. J. Rohlf, 1969. Biometry, 1st. ed. W. H. Freeman, New York.

Sokal, R., F. J. Rohlf, 1981. Biometry, 2nd. ed. W. H. Freeman, New York.

Van den Assem, J., F. Jachmann, P. Simbolotti, 1980a. Courtship behavior of Nasonia vitripennis (Hym., Pteromalidae): some qualitative, experimental evidence for the role of pheromones. Behavior 75: 301-307.

Van den Assem, J., M. Gijswijt, B. Nubel, 1980b. Observations on mating and courtship strategies in a few species of parasitic wasps (Chalcidoidea). Neth. J. Zool. 30: 208-227.

Van den Assem, J., F. Jachmann, K. DeJong, 1981. Courtship behavior of Nasonia vitripennis: head nodding, mouth-part extrusion, and pheromone discharge by abdomectomized males. Ent. exp. & appl. 30: 215-218.

Wallace, B., 1966. On the dispersal of Drosophila. Am. Naturalist 100: 551-563.

Whiting, Anna R., 1967. The biology of the parasitic wasp Mormoniella vitripennis (=Nasonia brevicornis) (Walker). The Quarterly Review of Biology 42: 333-408.

VITA

Robert Perry Steele

Born in Radford, Virginia, March 11, 1960. Graduated from Radford High School, Radford Virginia, June 1978. Attended Georgia Institute of Technology, September 1981 through June 1983. B.S., Davidson College, August 1983. Graduate Teaching Assistant in the Department of Biology, College of William and Mary, September 1983 through May 1985. Candidate for M.A. at the College of William and Mary, September 1983 through May 1985.