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Natural Selection in the Laboratory for a Change in Resistance by *Drosophila melanogaster* to the Parasitoid Wasp *Asobara tabida*

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The selection response of the polymorphic host D. melanogaster *(Meigen) to the braconid wasp* A. tabida *(Nees) is addressed. Cages of flies with and without wasps were initiated with a population of* D. melanogaster *that exhibited variation both in larval foraging behavior and in encapsulation ability. Encapsulation ability was measured as the proportion of parasitized larvae that produce a hardened capsule which encapsulates the wasp egg and ultimately kills the wasp larva. We determined whether the host population changed its encapsulation ability and/or its foraging behavior in response to the wasp. Both species were collected from a local orchard where* A. tabida *is the only wasp known to parasitize* D. melanogaster *larvae. The naturally occurring genetic polymorphism for rover and sitter larval foraging behavior in* D. melanogaster *is also found in this field population.* A. tabida*'s vibrotactic search behavior enables it to detect rover more frequently than sitter larvae. Rover larvae move significantly more while feeding than do sitter larvae. In this field population, rover larvae also show higher encapsulation abilities than do sitter larvae. Six cage populations, three without wasps and three with wasps, each containing an equal mixture of rover and sitter flies, were established in the laboratory and maintained for 19 fly generations. Selection pressure in the laboratory was similar to that found in the field population from which the flies and wasps were derived. We found that larvae from cages with wasps developed a significantly higher frequency of encapsulation than those reared without wasps. We were, however, unable to detect a change in larval movement (rover or sitter behavior) in larvae from cages subject to selection from wasps compared to larvae from cages containing no wasps. This may have resulted from a balance between two*

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selective forces, selection against rovers by the wasps' use of vibrotaxis, and selection for rovers resulting from their increased encapsulation abilities.

KEY WORDS: *Drosophila*; behavior; polymorphism; parasitoid wasp; host defense.

INTRODUCTION

Since parasites reduce the fitness of individual hosts, it has been proposed that they may act as a selective force to instigate evolutionary changes in the host population (Price, 1980). Although the host population may have certain defenses against a parasite which prevent unlimited exploitation, the parasite in response may evolve strategies which will overcome these defenses (Price, 1975). A continuing battle (arm's race) may persist as one species attempts to win over the other and, eventually, an equilibrium may be reached between the two species. Many researchers have attempted to model theoretically host-parasite interactions and reciprocal coevolutionary change (Slatkin and Maynard Smith, 1979; Anderson and May, 1982; Levin, 1983; May and Anderson, 1983).

Insect parasitoids are similar to parasites in that during their early life stages, they require a host to survive. However, a number of features set parasitoids apart from parasites (Doutt, 1959). First, parasitoids consume the tissues of their host, resulting in the death of the host. Second, they are free-living as adults. Third, they are more similar to their hosts than are parasites (Bouletreau, 1986), since insect parasitoids generally come from the same taxonomic class as their hosts and both partners have very similar generation times and reproductive rates. Parasitoids may also act as agents of selection, since when they are successful, they effectively reduce the fitness of a host to zero.

The evolution of host defenses may occur in a number of ways. First, many hosts employ measures of "concealment" and "avoidance" to escape detection by a host-searching parasitoid. Refuge-building by hosts as a means of concealment (Hawkins and Gross, 1992), the construction of accessory burrows by host wasps (Evans, 1966), an increase in larval digging (Carton and David, 1985), and decreased calling of male satellite crickets (Cade, 1975) are but a few examples where hosts have appeared to modify their behavior as a tactic for evading parasitoid detection. Second, once detected, hosts may employ morphological and behavioral defenses as a means of reducing the probability of oviposition by the parasitoid (for a review see Gross, 1993). Finally, once the parasitoid has oviposited, in the case of endoparasitoids, the host may elicit an immune response, such as encapsulation (Salt, 1970), which represents the last line of defense against the developing foreign egg. This process involves an aggregation of hemocytes around the egg, followed by a series of reactions

leading to melanization and cross-linking of proteins around the hemocytes to produce a hardened capsule. If the formation of the capsule is complete, the parasitoid is unable to emerge from the egg and the wasp larva dies from lack of oxygen and/or the inability to move and feed while the fly larva continues development (Salt, 1970). If the capsule is not complete, the wasp larva survives, hatches from the egg, and consumes the tissues of the host. Ultimately, the fly larva dies and a wasp emerges from the fly puparium. A genetic basis for variation in encapsulation ability in *D. melanogaster* has been detected (Carton and Bouletreau, 1985; Carton *et al.*, 1992).

Previous studies have investigated the interactions of parasitoids and *Drosophila* hosts. These studies, however, have examined the impact of selection in the laboratory using wasps and flies that were collected from different populations and which, therefore, had no previous history of interaction in the field (Bouletreau *et al.*, 1984; Bouletreau, 1986; Carton and Sokolowski, 1992). Furthermore, the flies used in these studies carried morphological genetic markers not found in natural populations; these markers allowed for rapid estimation of the response to selection. We have developed a model sytem which lends itself to studies of natural selection in the laboratory. In the present study, we used flies with orchard-derived genetic backgrounds and no morphological markers. We established population cages with hosts and parasitoids originated from the same orchard population and chose an orchard where *A. tabida* is the only parasitoid that parasitizes *D. melanogaster*.

At the site where these wasps were collected, a naturally occurring genetic polymorphism for larval foraging behavior in *Drosophila melanogaster* is found (Sokolowski, 1985; de Belle and Sokolowski, 1987; de Belle *et al.*, 1989). The *foraging* gene has two alleles, for^R (rover) and for^s (sitter), with the rover phenotype showing complete dominance over the sitter phenotype. Larvae with the "rover" phenotype travel significantly farther while feeding than those with the "sitter" phenotype. The searching behavior of *A. tabida* is well understood (Vet and van Alphen, 1985). This *Drosophila* larval parasitoid uses larval movement as a cue for host location (Sokolowski and Turlings, 1987). These authors predicted that *A. tabida* should be better able to detect rover compared to sitter larvae. Indeed, Kraaijeveld (1994) showed that in a single generation experiment *A. tabida* parasitizes rover larvae significantly more than sitters. In the present study we were interested in the long-term selection response of the polymorphic host *D. melanogaster* population to the wasp *A. tabida*. We initiated population cages, some with and some without wasps, which contained populations of *D. melanogaster* that exhibited variation in larval foraging behavior and in encapsulation ability. We determined (1) if the host population developed increased resistance to the wasp and (2) if the proportion of rovers relative to sitters decreased as a result of exposure to the wasp.

MATERIALS AND METHODS

Strains and Maintenance

Rover and sitter strains were originally derived as described by Pereira and Sokolowski (1993). Briefly, 500 *D. melanogaster* adult flies were collected from an orchard in the Toronto area in 1988. Significant differences in larval foraging behavior had been identified previously in larvae sampled from this site (Sokolowski, 1985). Flies were subsequently reared in the laboratory and the population was not allowed to go through bottlenecks. Twenty bottles with 300 flies per bottle were maintained for 1 year. Prior to the experiment flies from all bottles were mixed and the lengths of the foraging trails of 500 of their third-instar progeny larvae were measured (as described by de Belle and Sokolowski, 1987). Individual rover- and sitter-behaving male larvae were used to produce homozygous rover (for^R/for^R) and sitter (for^s/for^s) strains. Since *foraging* (*for*) had been localized to chromosome 2 at cytological position 24A3-5 (de Belle *et al.*, 1989, 1993), we crossed the sampled male flies to females from a chromosome 2 balancer stock [*In(2LR)SM1*, al^2 *Cy* cn^2 sp^2/*In(2LR)*bw^{v1}, ds^{33k} bw^{v1} (described by Lindsley and Grell, 1968)] which had been repeatedly backcrossed (10 times) to flies from the orchard population. Therefore, the resulting two rover and sitter strains used in this experiment carry genetic backgrounds similar to those of flies from the orchard population do not possess genetic markers and are homozygous with either a for^R or a for^s at the *for* locus. We verified this last condition with crosses to the deficiency strain $Df(2L)ed^{Sz}$, which uncovers *for* (de Belle *et al.*, 1989).

A. tabida was collected in August 1989 and 1990, from the same orchard as the flies (described above). Wasps were reared in the laboratory using wild-type *D. melanogaster* (a mixture of rover and sitter flies originally sampled from the orchard) as hosts. Wasps were maintained at 22 ± 1°C and a L:D 18:6 photocycle, with lights on at 0800. Strains of *D. melanogaster* were reared in plastic bottles on 45 ml of medium consisting of 5.0% dead yeast with sucrose, agar, and salts (standard mixture) at 25 ± 1°C and a L:D 12:12 photocycle with lights on at 0800. Rover and sitter strains were maintained separately for 1 year prior to the initiation of the selection experiment.

Population Cage Experiment

Initiation and Maintenance

Six dome-shaped plexiglass population cages were established (dimensions of each cage: length = 45 cm × width = 9.0 cm × height = 5.5 cm). Three cages with flies and wasps were designated treatment cages (T1, T2, and T3) and three cages with flies alone were control cages (C4, C5, and C6). Cages

were established with approximately 1000 flies from the rover and 1000 from the sitter strains. Food cups present in the cages contained a standard mixture of yeast–agar–sucrose medium. A thin coat (less than 1 mm deep) of 55% (w/v) dead yeast was spread on the surface of the medium to encourage flies to lay eggs and to promote larval foraging on the surface. All population cages were maintained at 22 ± 1°C in a L:D 18:6 photocycle with overhead illumination on at 0800 (to be called "standard conditions"). The fly populations were maintained in discrete generations and wasps were only present in T cages during the larval stage of fly development (see below). Wasps that emerged from each treatment cage were stored at 12 ± 1°C and a L:D 18:6 photocycle with sucrose and water.

Selection Regime

When caged flies were between 2 and 6 days old, they were provided with six fresh food cups containing 10 ml of medium. They were allowed to oviposit on the medium in these cups for 24 h. The cups were then removed and stored for 24 h after which time the second-instar larvae were at least 24 h old and susceptible to parasitism by this wasp (van Alphen and Drijver, 1982). The cups containing larvae were then introduced into another set of cages which contained either no wasps (C cages) or cages that contained female wasps (T cages) with previous oviposition experience on rovers and sitters. All females were mated and given previous oviposition experience for 24 h using 24-h-old wild-type host larvae (a rover and sitter mixture). While in the T cages, female wasps (between 6–12 days in age) were allowed to parasitize larvae for 48 h after which they were discarded. Flies and larvae from C cages were handled in an identical manner to those in T cages except that larvae from these cages were never exposed to wasps.

One week after the wasps were removed from the cages, flies began eclosing from the food cups. When these flies were 2–6 days in age, they were transferred to a clean cage with fresh medium and the procedure described above was repeated. Adult wasps emerged in the old cage approximately 1 week after the flies and were stored at 12°C until they were used in the next bout of selection. A given group of wasps was thus used every other fly generation. Several base populations of wasps were also reared on a wild-type population which contained a mixture of rovers and sitters. Wasps from these base populations were combined with those arising from the selection experiments. Together they comprised the wasps used for the next generation of selection. Wasps were randomly assigned to T cages. All T cages contained an equal number of female wasps during any one generation. Levels of parasitism are given in the results.

Densities of flies were estimated every generation as follows: the fresh

weight of 200 randomly sampled flies was measured from each cage and then all of the remaining flies were weighed as one large mass for each cage. This selection regime was maintained for 19 generations. An estimate of parasitism pressure by the wasps in the cages was made by calculating the percentage of wasp to fly emergence for each generation of selection. An estimate of parasitism pressure in the field was determined by collecting rotting pears from the orchard and counting all emerging wasps and flies over a one month period.

Path Length Tests

Changes in mean larval path length, a measure of the relative frequency of rover and sitter foragers, were measured using larvae randomly sampled from each cage every other generation. Larvae were collected as follows: after the first set of food cups containing freshly laid fly eggs had been removed (see above), a second set of food cups containing standard fly medium was introduced into each cage for another 24-h oviposition period. These food cups were removed from the cages and supplemented with approximately 50 ml of standard fly medium in petri dishes (14-cm diameter × 1.5 cm deep). Larvae were reared under standard conditions to the midthird instar and tested in the foraging assay (modified from de Belle and Sokolowski, 1987). We measured the distance each larva traveled while feeding on a circular yeast (52%, w/v) patch (8.5 cm in diameter) during a 5-min test period. One hundred larvae were tested from each cage every other generation.

Postselection Experiments

Larval Encapsulation Ability and Wasp Developmental Times

For each test, 30 early second-instar *D. melanogaster* larvae (±3 h in age) were placed in a dish (5.5-cm diameter × 1.4 cm deep) containing 10 ml of 3.2% agar, with a thin layer of 55% (w/v) dead yeast spread on top. A single experienced female wasp was introduced into each of these dishes. Once a wasp had oviposited in one larva, she was allowed an additional 4 h to parasitize the rest of the larvae in the dish. Control dishes of larvae, which were never exposed to wasps, were also initiated simultaneously to determine larval mortality rates in the absence of wasps.

At the end of the 4-h oviposition period, all larvae were removed from the dishes and placed in vials containing 10 ml of standard food medium. The insides of the vials were lined with sheets of acetate which provided an easily removable substrate on which the larvae could pupate. Vials containing the larvae were stored under standard conditions.

Eleven days later, eclosing flies were visually scored for the presence of a melanotic capsule in their abdomen. Flies with a capsule had survived parasitism

by the wasp by encasing the wasp egg with a thin layer of melanin (Carton and Kitano, 1981). Flies emerging without a capsule had not been parasitized. After all flies eclosed, the remaining pupae were transferred, with the aid of the acetate sheets, to "fresh" vials containing 5 ml of 3.2% (w/v) agar and incubated as described above. Nineteen days after the larvae had been parasitized, wasps began to emerge from the pupal cases. Wasps were subsequently collected twice a day at 0900 and 2100, sexed, and immediately killed. Egg-to-adult developmental times were determined only for those wasps which emerged within the next 12 days. This accounted for 94% of the total wasp emergence. The other 6% emerged significantly later and appeared morphologically abnormal and were discarded. Dry weights of individual wasps were determined after drying at 55°C for 2 days.

The presence of a dark melanotic capsule is more easily recognizable in a larva than in an adult fly, however, one might argue that ultimately it is the survival of the larva to an adult fly that is important, therefore, we provide two methods for estimating encapsulation ability. In the second method, larvae, rather than adult flies, were scored for the presence of an encapsulated egg. Dishes were set up exactly as described above except that *D. melanogaster* larvae remained in the dish and their food was supplemented with a yeast/water paste 2 days after exposure to wasps. Dishes with larvae were kept under standard conditions for 4 days in larger closed dishes (9.0-cm diameter and 2.4 cm deep) to minimize condensation. At this time larvae reached late third-instar and were dissected in *Drosophila* Ringer's solution (Ashburner, 1989) under a light dissection microscope. A dissected fly larva contained either (i) an encapsulated wasp egg, (ii) a living wasp larvae, or (iii) neither of the above (it was not parasitized).

Encapsulation ability for both experiments was determined by calculating the proportion of larvae or adult flies with an encapsulated egg out of the total number of larvae parasitized per dish. Parasitoid success was determined by calculating the proportion of wasp larvae or wasps that emerged out of the total number of larvae parasitized per dish. A measure of host mortality due to the parasitoid was estimated by subtracting the total number of dead larvae and pupae found in control dishes from the total number of dead larvae and pupae recorded in each of the test dishes. This value has been expressed as a proportion of the total number of individuals parasitized. All individuals were accounted for in each dish. For a more detailed explanation of the calculations, see Carton *et al.* (1989).

Encapsulation ability and wasp developmental times were measured on the rover and sitter strains which were used to initiate the population cages and for the treatment and control cage populations. Ten dishes (replicates) per strain were used with 2 control dishes for larval encapsulation experiments, 13 dishes per strain were used with 2 control dishes for the adult encapsulation experiments

on the rover and sitter strains, and 4 dishes plus 1 control dish per cage were used for the cage population estimates of encapsulation ability. For the cage populations, encapsulation abilities were determined one and two generations postselection for the larvae and adult experiments, respectively. The data from dishes in which less than 20% of the individuals had been parasitized were omitted (Mollema, 1988). This comprised 6% of the total dishes used.

Statistical Analyses

Repeated-measures analysis of variance (SAS Institute, 1985) was used to examine changes in mean path length of larvae sampled from treatment and control cages over time (GLM procedure). Since the assumption of compound symmetry was not met and correlations among the variables were not constant over time, the probability values were adjusted using the Greenhouse Geisser correction factor.

We examined differences in overall survival of *D. melanogaster* and *A. tabida* after parasitism for all larval comparisons using categorical analysis. Initially, we used chi-square analysis to examine whether differences in survival of these two species are apparent after accounting for differences in the general mortality of larvae following parasitism. To partition the variation in larval encapsulation ability due to the effects of treatment, cage, and dish, we used categorical analyses. Log-linear models were fit to the observed number of larvae that were classified into one of two categories. The larva (or fly) either died following parasitism or survived parasitism by producing a capsule. Likelihood tests were used to test the fit of those models to the data (BMDP, 1985). We tested a series of models, starting from the most saturated model, from which one interaction term at a time was removed until a model was found that no longer fit the data. We accepted the simplest model that provided a good fit to the data. To compare larval encapsulation ability of rovers and sitters, we performed a Student's t test on arcsine transformed proportion data (number of larvae surviving/number of larvae parasitized per dish).

All other postselection experimental data were analyzed as a two-level nested analysis of variance, with treatment as a fixed effect and cages, random and nested within treatment and vials, random and nested within cages (GLM procedure). All of these data, with the exception of one data set, met the assumptions of the analysis of variance and transformations were not required; mean wasp developmental times for males and females using larvae from T and C cages were log-transformed. Comparisons of densities of flies in T and C cages as well as comparisons of mean wasp developmental times of rovers and sitters were made using a one-level nested analysis of variance, with the top level fixed and the next level random.

RESULTS

Parasitism Levels

Mean fly densities (±SE) across all generations were 3954.3 ± 276.2 ($N = 51$) for T cages and 4571.8 ± 333.9 ($N = 51$) flies for C cages. This difference, which is nearly significant (nested ANOVA: $F = 5.96$, df = 1,4, $P = 0.07$), suggests that the wasps were reducing fly densities in the T cages. Parasitism pressure in the field population at the time when the wasps were collected was 16%. This was estimated from the ratio of the number of wasps/number of flies emerging × 100 in our field collections. Parasitism pressure in our laboratory experiment, expressed as a percentage, averaged 6.1 ± 1.5 ($\bar{X}$ ± SE) for cage 1, 7.6 ± 2.1 for cage 2, and 8.3 ± 1.8 for cage 3. Parasitism pressure for all cages did not change over the course of selection and the variation in selection pressure was similar for all cages.

Larval Encapsulation Ability

Rover larvae had significantly higher encapsulation abilities than did sitters (Table I), regardless of whether adults (Student's t test = 3.37, df = 23, $P < 0.01$) or larvae ($t = 2.98$, df = 12.7, $P < 0.01$) were scored. Recall that these rover and sitter strains had not been prior subjects in our selection experiments. Looked at in another way, the survival of *A. tabida* to an adult wasp was higher in sitter larvae than in rover larvae (Table II). This suggests that the wasp was more successful in sitter larvae, presumably because of this strain's relatively poor ability to encapsulate the wasp's egg. Differences in encapsulation ability

Table I. Encapsulation Abilities (%) ± SE of *D. melanogaster* Rovers and Sitters as Scored in Adult Flies and in Late Third-Instar Larvae When Parasitized by *A. tabida*[a]

Stage scored	Adults	Larvae
Rovers	18.6 ± 3.28 (12)	19.7 ± 3.06 (10)
Sitters	5.53 ± 1.04 (13)	10.5 ± 0.94 (10)

[a]Encapsulation ability is scored as the number of larvae known to have been parasitized that were able to encapsulate the wasp egg. The number in parentheses is the number of dishes of larvae tested (see methods).

Table II. Survival (%) of *A. tabida*, Scored as Adults, When Reared in Homogeneous Rover and Sitter *D. melanogaster* Strains[a]

	Rovers ($N = 210$)[b]	Sitters ($N = 288$)
Host mortality due to the wasp	30.26	27.96
Encapsulation ability	18.57	5.53
Adult wasps present	50.25	65.35

[a]Host mortality in the absence of the wasp was 3.33% ($N = 60$) for both rovers and sitters.
[b]Number of individuals tested.

and wasp emergence in rovers and sitters were highly significant ($\chi^2 = 22.68$, df = 2, $P < 0.001$). The developmental time or dry weight of wasps did not differ when reared in larvae from the rover or sitter strains (nested ANOVA). Mean wasp developmental times in days $\pm$ SE (N) were 22.83 $\pm$ 0.21 (66) and 23.14 $\pm$ 0.16 (118) in rover and sitter females and 21.16 $\pm$ 0.28 (29) and 21.70 $\pm$ 0.26 (51) in rover and sitter males, respectively. Mean wasp weights as micrograms $\pm$ SE (N) were 236.4 $\pm$ 5.1 (66) and 233.0 $\pm$ 4.6 (118) in rover and sitter females and 202 $\pm$ 7.0 (29) and 197.0 $\pm$ 5.4 (51) in rover and sitter males, respectively. Thus, wasp survivorship as a result of encapsulation ability differed between rover and sitter hosts. However, if a wasp egg was not encapsulated the weight of the wasp or its developmental time was not affected by which host it grew in.

We found significantly higher encapsulation abilities and wasp larva success in T compared to C cages at 19 generations of selection (Table IIIA; $\chi^2 = 10.18$, df = 2, $P < 0.01$) when larvae were scored and when emerging adults were scored (Table IIIB; $\chi^2 = 6.60$, df = 2, $P < 0.05$). These results suggest that the survival of *A. tabida* to adulthood was higher in larvae sampled from C cages than in larvae from T cages. The result of the log-linear analyses on the encapsulation data scored in larvae was that the simplest best-fit model showed that treatment (larva from control or treatment cages) had a significant influence on the ability of the larvae to survive parasitism by encapsulation and that there was a significant interaction between the influences of cage (T1, T2, T3, C4, C5, C6) and dish on larval survival ability (likelihood ratio for the simplest model that fit the data survival ability*treatment, survival ability*dish*cage: $\chi^2 = 14.50$, df = 11, $P = 0.21$). The hypothesis that the influence of the treatment on survival ability was zero was rejected (likelihood ratio for the interaction term survival ability*treatment: $\chi^2 = 9.00$, df = 1, $P = 0.003$). The simplest model for the encapsulation ability data scored as adults

Table III. Survival (%) of *A. tabida* and *D. melanogaster* (via Larval Encapsulation) After Parasitism of Larvae Sampled from Treatment and Control Cages[a]

(A) Scored as Larvae[b]	Treatment (N = 178)[c]	Control (N = 202)
Host mortality due to the wasp	13.88	11.02
Fly larvae with capsule (encapsulation ability)	38.87	22.89
Surviving wasp larvae	47.25	66.09
(B) Scored as emerging adults[d]	(N = 203)	Control (N = 194)
Host mortality due to the wasp	30.18	29.09
Adult flies with capsule (encapsulation ability)	36.71	26.07
Surviving adult wasps	31.24	42.78

[a]Note that total wasp mortality includes fly larvae which died as a result of parasitism and does not include those larvae which were able to encapsulate a wasp egg successfully.
[b]Host mortality in the absence of the wasp was 1.11% (N = 90) and 4.44% (N = 90) for treatment and control cages, respectively.
[c]Number of individuals tested.
[d]Host mortality in the absence of the wasp was 4.44% (N = 90) and 2.27% (N = 88) for treatment and control cages, respectively.

showed that treatment and cage had significant effects on the ability of flies to survive parasitism (likelihood ratio for the model survival ability*treatment, survival ability*cage: $\chi^2 = 16.99$, df = 20, $P = 0.65$). The hypothesis that the influence of the treatment on survival ability was zero was again rejected (likelihood ratio for the interaction term survival ability*treatment: $\chi^2 = 5.28$, df = 1, $P = 0.02$). Overall, these results suggest that there were significant differences in the response of larvae from the treatment (with wasp) and control (no wasps) cages to parasitism by the wasp: larvae from treatment cages show a significantly higher encapsulation ability than those from control cages.

Developmental time or dry weight of wasps did not differ when reared in larvae from the control or treatment cages (nested ANOVA). Mean wasp developmental time in days $\pm$ SE (N) were 23.91 $\pm$ 0.23 (47) and 23.63 $\pm$ 0.24 (53) for females and 21.91 $\pm$ 0.36 (23) and 21.69 $\pm$ 0.25 (35) for males from treatment and control cages, respectively. The mean dry weights of wasps as micrograms $\pm$ SE (N) were 224.3 $\pm$ 5.9 (47) and 239.8 $\pm$ 5.7 (53) for females and 221.9 $\pm$ 9.0 (23) and 201.9 $\pm$ 6.5 (35) for males from treatment and control cages, respectively.

Selection for Path Length

Figure 1 shows the mean path lengths of larvae from T and C cages over a period of 19 generations. There was an overall increase in path length (the "apparent" frequency of rover) but this was true in both treatment and control cages. Repeated-measures analysis of variance revealed no significant treatment effect ($F = 0.29$, df = 1,4, $P = 0.62$) and a significant time effect ($F = 10.93$, df = 8,32, $P = 0.001$). The increase in mean path length observed in the first seven generations was likely due to the increase in the number of for^R/for^s heterozygotes (rover phenotype), resulting from matings between the rover and the sitter homozygotes used to establish the population cages. By generation 7, what appeared to be a stable equilibrium in mean path length in all cages was reached. The nonsignificant interaction term in the repeated-measures analysis indicated that there was no significant difference in mean path length between T and C cages over time ($F = 0.65$, df = 8,32, $P = 0.59$).

DISCUSSION

The evolution of an increase in host resistance has been predicted (Bouletreau, 1986) since, first, selection should favor those hosts that are able to demonstrate encapsulation ability and, second, selection should also favor those parasitoids which are able to overcome the defense reaction of the hosts

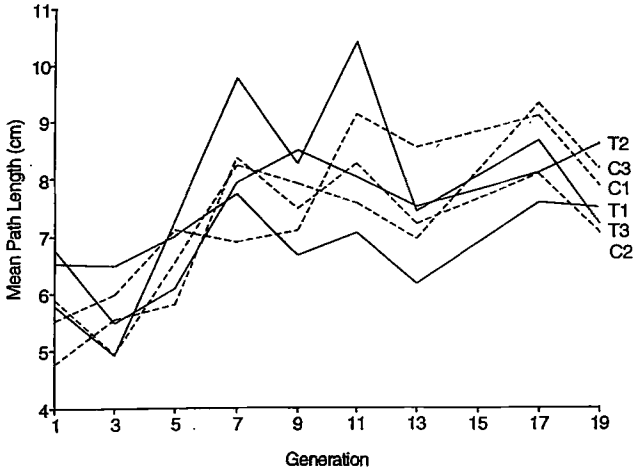

Fig. 1. Mean larval path length (cm) of 100 larvae measured for three treatment (T1, T2, and T3) and three control (C1, C2, and C3) cages every other generation for 19 generations of selection. Solid lines represent treatment cages and dashed lines represent control cages.

and thereby increase the selective pressure exerted on the host (van den Bosch, 1964; Salt and van den Bosch, 1967; Messenger and van den Bosch, 1971). In the present study we found support for the first prediction. We found an increase in encapsulation ability in flies sampled from treatment cages compared to those flies sampled from control cages.

Previous studies have demonstrated a genetic basis for variation in encapsulation ability using isofemale lines of flies collected from nature (Carton and Bouletreau, 1985). Carton *et al.* (1992) determined that the ability to encapsulate parasitoid eggs appears to fit a one-gene Mendelian autosomal model of inheritance, with the high encapsulation ability line showing complete dominance over the low one. To date, the encapsulation gene has not yet been localized to a specific autosome.

It is uncertain at what level the "encapsulation ability" protein might act to mediate the host immune process. Some evidence suggests that the "encapsulation ability" gene may be important at the level of wasp egg recognition, and not in the synthesis of melanin. Vass *et al.* (1993) found that a strain of *D. melanogaster* which showed low larval encapsulation when parasitized by *Leptopilina boulardi* showed a high encapsulation ability when parasitized by *A. tabida*. Another strain showing a high encapsulation ability against *L. boulardi* showed a similar high response against *A. tabida*. Both of these strains were found to have similar levels of mono- and diphenol oxidases, key enzymes in melanin production indicating that the point of control may not be at melanin synthesis but rather involves the initial recognition process. This is not clear, however, since the ability to encapsulate is a complex interaction between the host and the wasp and it may have been that *L. boulardi* was simply able actively to suppress the host cellular response in the low encapsulation line.

The process of larval encapsulation as a host defense reaction involves a number of considerations including the condition of the host, the ability of the wasp to overcome the host's defenses and the extent of the interaction between the wasp and its host. Variability in the ability to encapsulate wasp's eggs suggests that there may be a cost associated with this process later in life. Precursors involved in the synthesis of melanin, for instance, are also used in the formation of the puparium (Anderson, 1985). Thus, larvae which are able to overcome parasitism successfully may suffer abiotic stresses such as those involved with desiccation resistance (Mollema, 1988). Furthermore, Carton and David (1983) have found that there are indeed fitness consequences associated with capsule formation; relative to control flies without a capsule, flies possessing a capsule were generally smaller and females produced fewer offspring.

After 19 generations of selection by *A. tabida* we did not find a decrease in the frequency of the rover morph in treatment cages. This may have resulted from differences in encapsulation abilities between rovers and sitters. In our populations, rovers show a significantly higher encapsulation ability relative to

sitters when they are parasitized by *A. tabida*. In other words, selection *against* rovers which are more likely to be attacked by *A. tabida*, who use vibrotaxis in host detection might be balanced by selection *for* rovers due to their enhanced encapsulation abilities.

The observed relationship between encapsulation ability and larval foraging behavior could be the result of either genetic pleiotropy (the products of both genes may be involved in the same biochemical pathway) or genetic linkage. It is also tempting to speculate that the neurotransmitter dopamine, which is an intermediate in melanin synthesis, might account for the observed difference in foraging behavior. Genetic localization and cloning of the "encapsulation ability" gene will help us understand the relationship between *foraging* and encapsulation ability.

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