

DISPERSAL BEHAVIOR OF THE LARVAL TENEBRIONID BEETLE ZOPHOBAS RUGIPES

WALTER R. TSCHINKEL

Department of Biological Science, Florida State University, Tallahassee, Florida 32306

(Accepted 2/6/78)

In the laboratory, crowding inhibits pupation of larvae of *Zophobas rugipes* (Coleoptera: Tenebrionidae). Pupation-ready larvae must disperse away from high-density populations in order to find an undisturbed pupation site. In the laboratory, the pupation chamber is defended against intruding larvae, and the success of occupants in preventing intrusion increases with isolation up to 96 h. Thereafter the larvae begin to enter the pharate pupal stage. Over a challenge period of 5–10 h occupant success declines to a plateau but remains above the randomly expected value for 24 h, provided the occupants have been isolated at least 12 h. Dispersal and single occupancy of chambers are due to fighting between occupant and challenger. The major components of the behavior are "shoveling" with the head, opening mandibles, biting the opponent, and violent side-to-side thrashing of the body. All these components increase rapidly during the first 24 h of isolation. Among mature larvae, success in chamber defense is not affected by relative weight (650–750 mg vs. 850–950 mg) or relative age (11–12 mo vs. 14–15 mo). Success is decreased but still significantly nonrandom if occupants are removed from chambers and forced to redisperse along with the challengers. Success is greatly increased if larvae are selected for success in a prior experiment. The ability to defend a pupal chamber successfully thus appears to be an intrinsic property of the larvae. Immobilized larvae are unable to prevent intrusion into their chambers, indicating that single occupancy results from active defense. Pharate pupae and pupae have a limited capacity to exclude intruders from their chambers. Immobilized larvae are subject to heavy cannibalism by active larvae.

INTRODUCTION

Dispersal as a response to crowding is common among insects. The crowding of aphids causes the production of alatae which fly to other sites (Lees 1961). High population density causes first instar larvae of the gypsy moth, *Porthetria dispar*, to disperse aerially on silken threads (Leonard 1970), and a similar mechanism operates in the mite, *Metatetranychus* (Marle 1951). The phase change of the migratory locusts at high densities is also essentially a dispersal response, for the gregarious phase adults disperse from their home areas in large swarms (Kennedy 1956).

Among tenebrionid beetles of the genus *Tribolium*, the dispersal of larvae

has received less attention than that of the adults. Naylor (1965), in the course of experiments on adult dispersal in relation to larvae, found that the larvae themselves had a sporadic and density-dependent tendency to disperse from the highly populated vials. Likewise, larvae in the complex habitats created by King and Dawson (1973) leave regions of high larval density to pupate in warmer, less dense regions. Ghent (1966) reports similar movement of "prepupae" in *T. confusum*. Perhaps because of the small size of *Tribolium*, all of these studies have dealt only with the outcome of dispersal and not with the behavior of the dispersing individuals. The proximate causes of dispersal are therefore obscure. Larger species of tenebrionids, such as *Zophobas rugipes*, are more suited for the study of dispersal behavior.

Larvae of *Z. rugipes* often occur under crowded conditions in caves where they feed on guano or other organic detritus. Like many other tenebrionid larvae, those of *Z. rugipes* are noteworthy in that crowding directly inhibits metamorphosis (Tschinkel and Willson 1971). Larvae maintained in crowded cultures will die of old age after 18–24 mo without ever transforming to the adult. Unlike those in lab cultures, larvae in nature can escape crowding by dispersal, and Tschinkel and Van Belle (1976) have shown that, as larvae grow, their distribution changes from aggregation to strong overdispersion, and that this change parallels an increase in the tendency to pupate upon isolation. The increasing overdispersal can therefore be viewed as adaptive in that it causes mature larvae to find undisturbed sites for pupation.

In the laboratory, larval dispersal is measured by allowing a number of larvae to assort themselves from a central arena into a number of identical chambers approximating pupal chambers (Tschinkel and Van Belle 1976). Observations during such experiments indicated that the repellent force causing the overdispersal of mature larvae was fighting between chamber occupants and intruders. Larvae appeared to compete for the chambers by combat, and this competition intensified as the ratio of larvae to chambers increased. These observations stimulated the present investigation of the role of behavior in the overdispersal and the effects of isolation time, larval age, and larval weight on larval success in chamber defense.

MATERIAL AND METHODS

All dispersal experiments were carried out in a $\frac{3}{4}$ -inch-thick, 55-cm-diameter circular Plexiglas "dispersarium" with a concentric 31-cm arena (see Tschinkel

and Van Belle 1976). The larvae were added to the central arena and allowed to distribute themselves among 26 identical, individually accessible, 4-cm chambers in the Plexiglas ring. The experiments were run under red light and chamber occupancy was checked at intervals. Because the chambers approximate pupal chambers the larvae prefer them to the arena. Since most dispersal experiments tested the relative ability of larvae of two competing groups to occupy chambers, one group was always identified with small spots of aluminum paint. The group marked was alternated among replicates.

Chamber defense behavior was studied in a 15 × 9-inch "encountorium" of $\frac{3}{4}$ -inch-thick Plexiglas. By opening gates, larvae in a central arena could be given individual access to any one of 14 identical 4-cm chambers, each containing one larva. The behavior which resulted from such encounters between chamber occupants and challengers from the arena was coded on a Rustrak eight-channel event recorder. All encountorium experiments were carried out under room light.

Statistical analysis used either a mixed-design or a factorial analysis of variance, as appropriate. Relative success of occupants and challengers was also tested with a Yate's Corrected χ^2 .

EXPERIMENTS AND RESULTS

THE EFFECT OF ISOLATION TIME ON SUCCESS OF CHAMBER DEFENSE

Twenty-six larvae aged 12–14 mo and weighing between 850 and 950 mg were selected from a crowded mass culture, placed into the arena of the dispersarium, and allowed to disperse among the 26 chambers (these larvae were called "occupants"). After a certain time ("isolation time"), a second group of 26 larvae (the "challengers"), of similar weight and directly from the same culture, was

added to the arena and allowed to disperse. The number of occupants and challengers in each chamber and in the arena was noted at successive $\frac{1}{2}$ -h intervals for the first 8 h and the last 4 h of a 24-h period. The periods of isolation before addition of challengers were 0, 2, 12, 24, 48, 72 and 96 h, and two replicates were run for each isolation time.

A larva which was alone in its chamber was defined as being successful in chamber defense, while a larva which shared its chamber with another or resided in the central arena was considered unsuccessful. Since this experiment was designed to assess the effect of isolation time on the success of chamber defense, the index of most interest here is the proportion of successful larvae which

were occupants (occupant success index). This index was calculated for each observation by dividing the number of successful occupants by the total number of successful larvae. The proportions were transformed to $\arcsin \sqrt{\%}$ to normalize them. A three-point moving average was calculated for each replicate, and the average of these averages was plotted against isolation time and time-since-challenge (fig. 1). An $\arcsin \sqrt{\%}$ value of 45 indicates that half of the successful larvae were occupants and is the value expected if prior occupancy gave no advantage to a chamber occupant, i.e., if the distribution were randomly determined.

Figure 1 shows clearly that the success of chamber defense among occupant

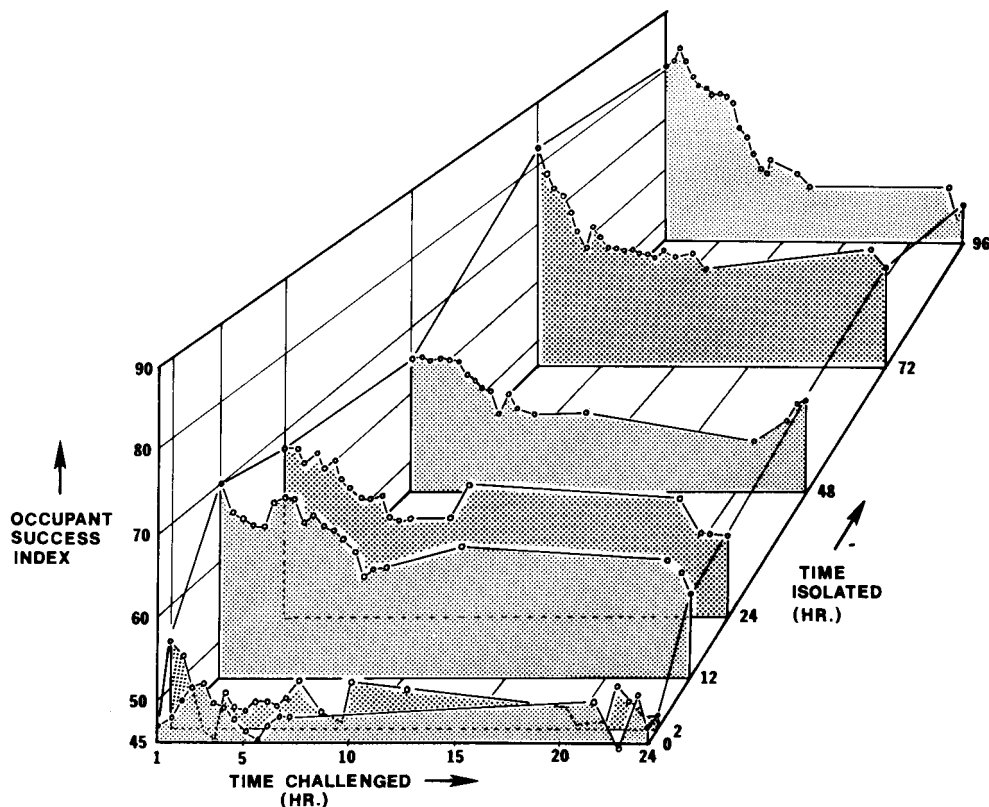


FIG. 1.—Occupant success index as a function of isolation time and challenge time. Each curve is based on the mean of two replicates. A value of 45 indicates that occupants made up half of the successful larvae, the value expected on the basis of chance alone.

larvae increases as isolation time increases, and that within each isolation time success decreases as challenge time increases. Both of these effects were shown to be significant (analysis of variance; $P < .02$, $< .01$, respectively; table 1). Furthermore, increasing isolation time decreases the negative effect of challenge time on occupant success (table 1, interaction isolation time \times challenge time). Nevertheless, even after 24 h of challenge, the occupants retain a significant margin of success provided they had been isolated for at least 12 h. The pattern within each isolation time is quite consistent—the occupants' share of success gradually declines over the first 5–8 h and remains more or less stable till the end of the 24-h period. During this initial period, fights are common within chambers, and some challengers are always successful in dislodging an occupant.

The advantage of isolation to the occupants seems to accrue rapidly. After

2 h of isolation, success index for occupants during the first 2 h of challenge is already significantly higher than randomly expected ($P < .05$, χ^2 test). The initial advantage of occupants remains approximately stable from 12 to 48 h of isolation and then peaks at 72 h. The ultimate advantage after 24 h of challenge follows a similar pattern, with the addition of a decline from 72 to 96 h. This decline may be due to the fact that the occupants enter the pharate pupal stage and thereby become quiescent and less able to defend their chambers. Some of these larvae become temporarily reactivated during the initial stages of challenge, accounting for the peak at 2 h in the 96-h isolation group.

The mean χ^2 value for the ratio, successful occupant:successful challenger (expected ratio 1.0) for the first 2 h of challenge, the second 2 h, and the last 4 h confirms these conclusions. As isolation time increases up to 12 h, the χ^2 value increases rapidly, stabilizes through 48 h,

TABLE 1
P-VALUES RESULTING FROM ANALYSIS OF VARIANCE OF
FACTORS AFFECTING CHAMBER DEFENSE

EXPERIMENT AND FACTOR	P			
	Occupant Success Index	Total No. in Chambers	Mean of Occupants Sharing	Mean of Challengers Sharing
Isolation time:				
Isolation time...	.02 incr.	.005 decr.- incr.	.10	.10
Challenge time..	.001 decr.	.001 decr.	.005 decr.	.001 decr.
Interaction.....	.01	NS	NS	NS
Age (simultaneous):				
Age.....	NS	NS	NS	NS
Challenge time..	NS	.001 decr.	.001 decr.	.002 decr.
Interaction.....	NS	.05	NS	.05
Age (24-h isolation):				
Age.....	NS	NS	NS	NS
Challenge time..	.002 decr.	.02 decr.	.05 decr.	.02 decr.
Interaction.....	NS	NS	NS	NS
Weight				
Weight.....	NS	NS	NS	NS
Challenge time..	.001 decr.	.001 decr.	NS	.001 decr.
Interaction.....	NS	NS	NS	NS

NOTE.—A larva is considered successful when it is the only larva in a chamber. Occupant success index is the proportion of successful larvae which were occupants, transformed to $\arcsin \sqrt{\%}$. All other data were analyzed without transformation. Where there is a significant effect, its direction is indicated next to the P -value. Incr. = increase; decr. = decrease.

peaks at 72 h, and declines at 96 h. With a single exception, the χ^2 values for the first 2 h are always highest, the second 2 intermediate, and the last 4 lowest. This results from the gradual erosion of successful occupants during the challenge period.

The total number of larvae in chambers (regardless of success) also reflects the course and intensity of competition for chambers (table 1; fig. 2). Since there are always twice as many larvae (52) as chambers, and since chambers are preferred over the arena, increased competition results in smaller total numbers of larvae in chambers and more in the arena (table 1; fig. 2a). Thus, the total number of larvae in chambers declines to a minimum for 24 and 48 h of isolation and increases again for the longest isolation periods. Competitiveness of occupants is inversely related to the total in chamber. Furthermore, the total in chamber declines over each 24-h challenge period as competition proceeds. Initially, larvae in the arena are predominantly challengers unable to displace a defending larva, but occupants are increasingly displaced into the arena as challenge continues.

Figure 2a shows that the changes in the total in chambers with isolation time are almost entirely due to the changing number of challengers in chambers. The total number of occupants in chambers is independent of isolation time, though it declines consistently during each 24-h challenge period. Except at 0- and 2-h isolation, there are always fewer challengers than occupants in the chambers. The dip in total larvae in chambers at intermediate isolation times is due almost entirely to the smaller number of challengers able to enter chambers. This is because as isolation time increases, occupants defend more effectively and prevent more challengers from entering the chambers.

Approximately the same trend is exhibited by the number of larvae in chambers but sharing with at least one other larva of either group (fig. 2b, table 1). The number declines to a minimum after 24 and 48 h of isolation and increases again at 72 and 96 h (fig. 2b) ($P < .10$). More importantly, figure 2b and table 2 show clearly that for each isolation time, while the number of sharing occupants declines somewhat over the 24-h challenge period ($P < .005$), the number of sharing challengers is initially much higher and declines over 24 h ($P < .001$) to a level comparable to that of the occupants. This is the result of both the general decrease in the total number of larvae in chambers (fig. 2a) and the proportion of successful larvae which are occupants (fig. 1).

CHAMBER DEFENSE BEHAVIOR

Defensive behavior was studied in an encounterium in which chamber access could be controlled. Each chamber contained an occupant which had been isolated for a specific period. Encounters were observed for 5 min from the first contact. Challengers were always fresh from the stock culture. Fourteen occupant-challenger pairs were sequentially observed in each of two replicates. Both occupants and challengers were from the same 13-14½-mo-old culture and weighed between 850 and 950 mg.

The four most common behavioral acts and exit from the chamber were recorded for each occupant and each challenger in all encounters. These four acts were (1) opening the mandibles, often for long periods; (2) biting the other larva, occasionally causing bleeding or even severing an appendage; (3) pushing the head under the opponent larva from the side and rapidly and forcefully lifting the head and anterior body by dorsal flexure and leg extension

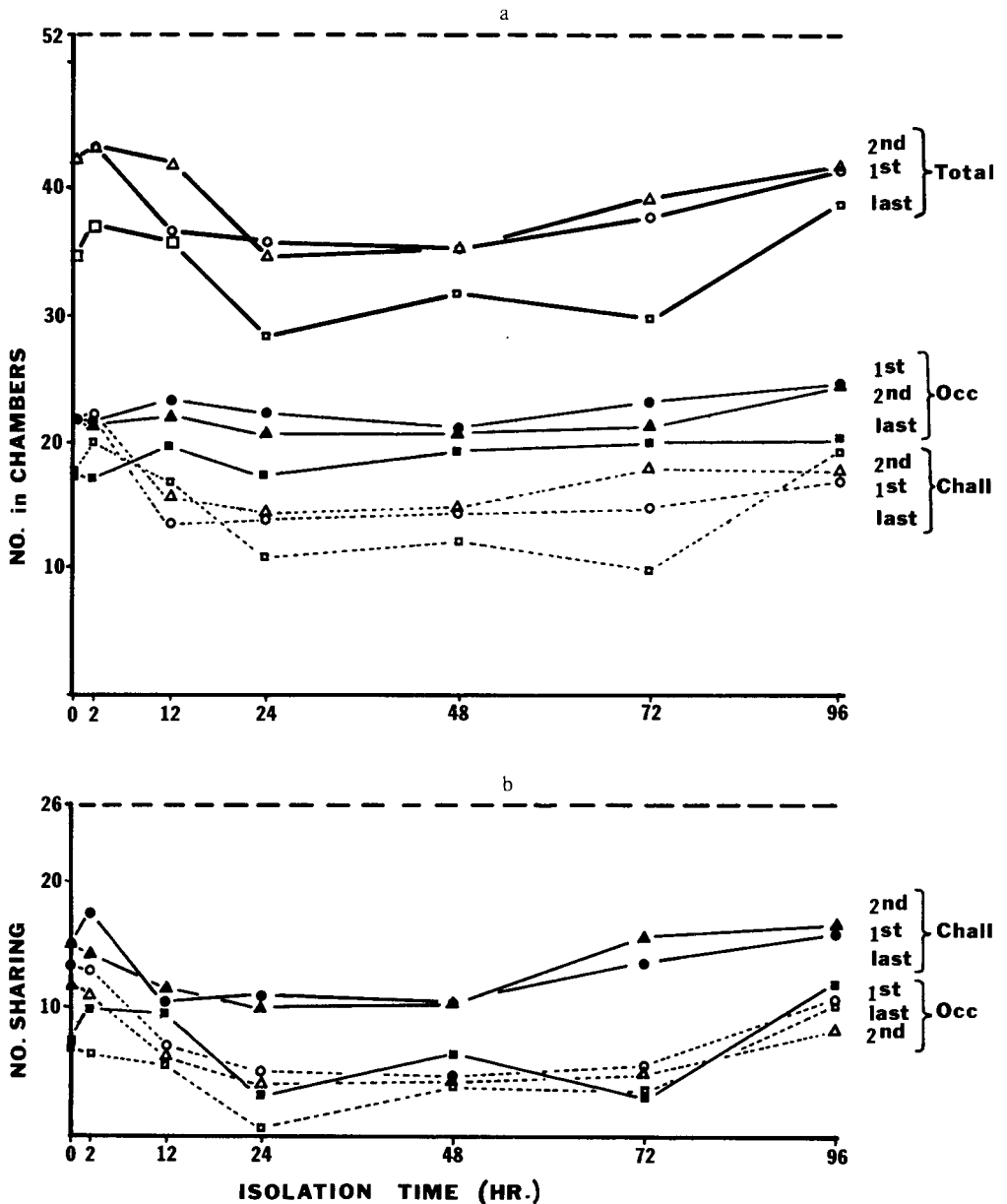


FIG. 2.—*a*, Total number of larvae, occupants, and challengers in chambers as a function of isolation time. Curves are plotted as means for the first 2, the second 2, and the last 4 h of the challenge period. There are 52 larvae in all—26 occupants and 26 challengers. *b*, Mean number of challengers and occupants sharing chambers as a function of isolation time. Curves are plotted as means for the first 2, the second 2, and the last 4 hours of the challenge period. Symbols: circles = first 2 h; triangles = second 2 h; squares = last 4 h.

(head shoveling); (4) thrashing the body from side to side, once to several times in rapid succession. Additional behavioral acts which were not recorded were increased locomotion, either forward or backward, and raising the head and anterior body off the substrate.

Increasing periods of isolation correlate with a rapid increase in the frequency of occurrence of these selected behaviors (fig. 3). After 2 h of isolation, occupants are already much more aggressive than challengers, and this aggressiveness continues to increase for at least 24 h. Challengers never show a high incidence of any of the aggressive behavioral acts.

The most common behavior among occupants at all isolation times is head shoveling which, after 24 h of isolation,

averaged about 1.8 per larva per minute. Biting was the second most common act, followed by thrashing.

Challengers did not exit from the chambers significantly more frequently than occupants. Perhaps the 5-min observation period is not long enough to assess the effect of occupant behavior on exit rate of challengers.

THE EFFECT OF LARVAL WEIGHT ON CHAMBER DEFENSE

Larvae 11–13 mos of age were selected to fall into two weight groups—a heavy group weighing between 850 and 950 mg and a light group weighing between 650 and 750 mg. The age of the larvae varies by 2 mo because of the time required to run the experiment, but groups of larvae tested against one another were always of

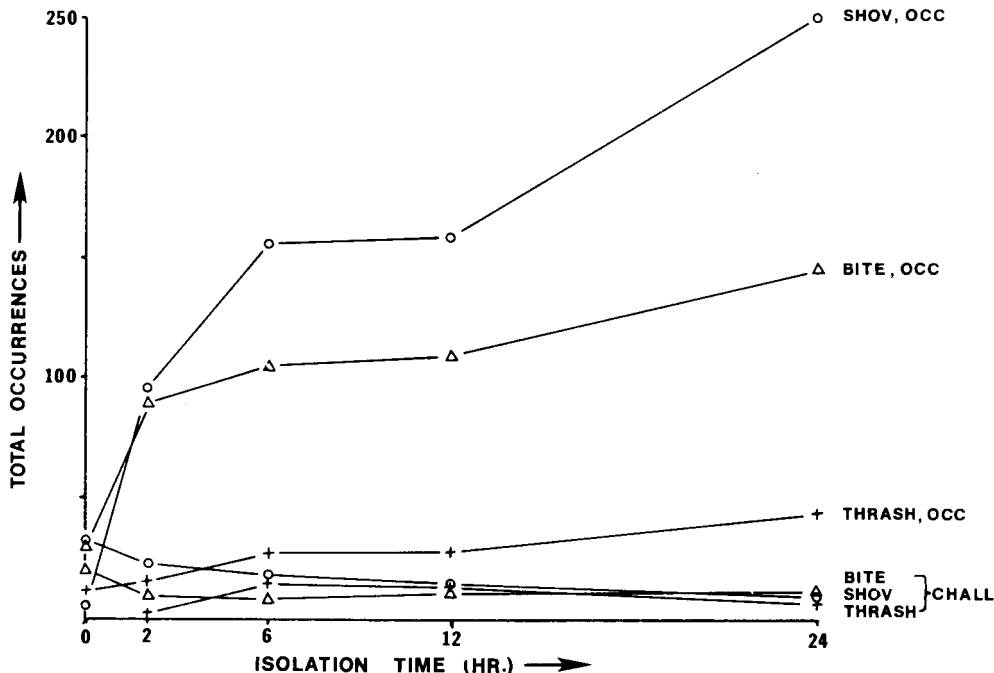


FIG. 3.—Total occurrence of three behavioral acts characteristic of chamber defense, in relation to isolation time of occupants. *Occ* = occupant; *chall* = challenger; *shov* = head shoveling; *bite* = biting; *thrash* = violent lateral thrashing of whole body. Totals are the sum of the encounters between 28 pairs of occupants and challengers.

the same age. Each weight group was used in turn as occupants isolated in the dispersarium for 24 h and then challenged for 24 h with the same or other weight group. The number of occupants and challengers in each chamber was noted every half hour for the first 8 h and the last 4 hs of the 24-h challenge period.

Analysis of variance of four dependent variables showed that weight had no effect on any measure of the success of chamber defense (table 1). In other words, heavy or light occupant larvae defend their chambers with equal effectiveness against either heavy or light challenging larvae.

The initial advantage held by occupants upon first addition of the challengers gradually declines during the first 5–8 h of the challenge period (table 1), just as it does in all such experiments, but even after 24 h the occupants retain some advantage.

It should be noted that the larvae in these experiments are all fairly old and all have a strong tendency to pupate. In other experiments with much younger larvae in mid-growth phase, weight does have an effect on chamber defense. For example, when 52 randomly selected larvae from a 4–5-mo-old culture are added to the dispersarium for 3 days, the successful larvae weighed significantly more than the unsuccessful (307 mg, SE 27 vs. 211 mg, SE 11, respectively). Upon isolation, the successful larvae resulted in twice the proportion pupating than did the unsuccessful (65% vs. 31%). The proportional differences in weight among these growth-phase larvae are much greater than among the mature larvae in the experiments, and it is not possible to rule out an effect of such large differences among mature larvae, although they rarely exist.

EFFECT OF LARVAL AGE
ON CHAMBER DEFENSE

Larvae of the same weight (850–950 mg) but two different ages competed against one another in these experiments. An old group consisted of 14–15-mo-old larvae, a young group of 11–12-mo-old larvae. Both old and young larvae are sufficiently old to show a strong tendency to pupate, and differences in ability to defend chambers are not the result of being too young to pupate. Each experiment was conducted in two replicates in which either the old or the young group of 26 occupants was isolated in the dispersarium for 24 h and then challenged with 26 either young or old challengers for another 24 h. Chamber occupancy was recorded as in previous dispersarium experiments.

As in previous experiments, the initial advantage held by occupants regardless of age is eroded over the initial challenge period to reach a more or less stable plateau (table 1). Analysis of variance showed no significant effect of age on any measure of the success of chamber defense (table 1). However, young larvae seemed to be stronger competitors for chambers, since old occupants facing young challengers consistently showed less success while young occupants pitted against old challengers defended their chambers quite as successfully as did each age group facing same-age challengers. Unfortunately, statistical analysis indicates that these effects are marginal ($P < .10$).

The effect of larval age was also tested by simultaneous addition to the dispersarium of the pairs of larval groups (old-old; old-young; young-young), so that none of the 52 larvae enjoyed the advantage of an isolation period. Under these circumstances, the proportion of successful larvae which belonged to

either age group never differed significantly from random (χ^2) at any time during the 24-h experiment. The total number of larvae in chambers, however, was initially higher than in the 24-h isolation experiment and fell strongly over the challenge period as individual larvae established themselves successfully in chambers (table 1). A similar trend can be seen in the number of larvae sharing chambers (table 1). Here too there is no significant difference among the age groups.

It thus appears that among mature larvae moderate differences in age have no significant effect on the ability to win and defend chambers, although some trends are evident.

SUCCESS AS AN INTRINSIC LARVAL PROPERTY

The advantage of isolation time to successful chamber defense could be site-dependent or due to changes in the larva itself. In order to test how much of the defense advantage was intrinsic to the larvae, the 26 occupants were removed from the dispersarium after 24-h isolation and mixed with 26 challengers in a box (all larvae were 9–10 mo old; 850–950 mg; 4 replicates). All 52 were immediately dumped into the center of the dispersarium. If the occupants' defense advantage was due to an intrinsic change, then these larvae should compose more than half of the successful larvae, even though they dispersed simultaneously with the challengers.

The results show that the occupants retain a significant margin ($P < .05$; χ^2) of success despite having to disperse again together with the challengers (fig. 4). In comparison with an experiment in which all conditions were similar but the occupants were not removed from chambers (24-h experiment, isolation time series; fig. 4, control), it is clear that some advantage is lost upon removal and

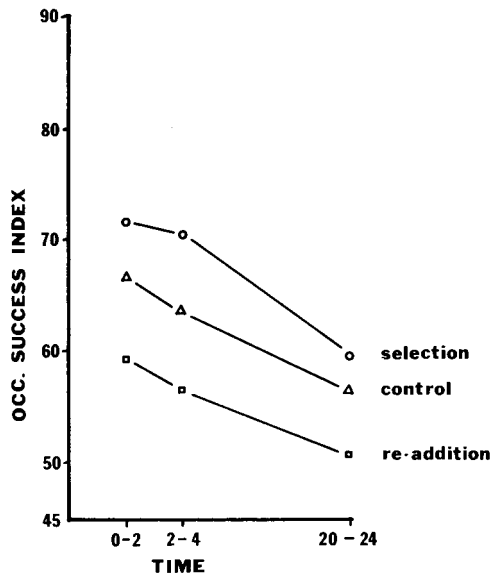


FIG. 4.—Occupant success index for the removal-readdition, selection, and control experiments. Control consisted of occupants isolated 24 h followed by addition of challengers. See text for details. The differences are not quite significant by analysis of variance because of the high variability of the re-addition replicates.

redispersal, for the success is always higher in the experiment in which occupants were not removed. These control larvae retained a significant margin of success even after 24 h of challenge ($P < .05$; χ^2), but the redispersed occupants did not. Occupant success index and challengers sharing both decreased significantly with challenge time (analysis of variance), again indicating intolerance on the part of occupants. Total in chambers and occupants sharing do not change significantly.

It thus appears that at least a portion of the advantage accruing to occupants during isolation is intrinsic to them. It seems likely that the decreased success upon redispersal is due to the difficulty of regaining a chamber which may in the meantime have been occupied by one to several larvae, including other equally motivated occupants.

If successful defense results from intrinsic larval properties, then larvae which were successful in one experiment should have a much larger chance of success in the next experiment, and unsuccessful larvae from the first should be less likely to succeed in the second.

Two dispersariums were therefore set up, each with twice as many larvae as chambers. After 24 h, 26 successful and 26 unsuccessful larvae were selected and weighed individually. All 52 were then added simultaneously to the center of an empty dispersarium and allowed to disperse for 24 h. This experiment differs from the previous in that there are no occupants and challengers, only successful and unsuccessful larvae, all of which have been in the dispersarium for 24 h.

The larvae selected for success in the first phase of the experiment retained a large margin ($P < .01$) over those selected for lack of success (fig. 4, *selection*), and this difference was still very significant ($P < .05$, χ^2) after 24 h. When the success index in the selection experiment is compared with those in the readdition experiment and the control (24-h isolation), it can be seen that selection has greatly increased success (fig. 4). The two experiments are not strictly comparable since, in the former, selec-

tion was not random and competition during the first phase was among 26 larvae while in the latter it was among 52 randomly selected larvae, all of which had been in a dispersarium for 24 h. Analysis of variance for these three experiments showed treatment (readdition, control, selection) to have a significant effect on total in chambers and occupant sharing, indicating that treatment affects larval intolerance (table 2). Occupant success index or challengers sharing were not significantly affected, probably because of the large variability of the readdition replicates. Nevertheless, the comparison reiterates the point that success and intolerance are intrinsic to the larvae.

The body weight of the larvae selected for success was significantly greater than those selected for lack of success (table 3). When these larvae were isolated for pupation after the dispersarium experiment, the successful larvae pupated significantly more rapidly than the unsuccessful ones (table 3, $P < .05$, Student *t*-test). The ability to defend a chamber successfully is thus correlated with greater body weight and increased speed of pupation. The unsuccessful larvae had of course spent less time without disturbance because they shared

TABLE 2
COMPARISON OF READDITION, CONTROL, AND SELECTION EXPERIMENTS

	Occupant Success Index	Mean of Total No. in Chambers	Mean of Occupants Sharing	Mean of Challengers Sharing
Factor: Levels of Significance ^a				
Treatment	NS	< .05	< .05	NS
Challenge time	< .001	< .02	< .05	< .002
Experiment: Ranking of Dependent Variables ^b				
Selection	1	2	2	2
Control	2	3	3	3
Readdition	3	1	1	1

^a *P*-values resulting from analysis of variance.

^b 1 = highest, 3 = lowest.

TABLE 3

SELECTION OF LARVAE FOR SUCCESSFUL AND UNSUCCESSFUL CHAMBER DEFENSE:
RELATION OF BODY WEIGHT AND DAYS-TO-PUPATION TO SUCCESS

	MEAN DAYS TO PUPATION (+SD)			MEAN BODY WEIGHT (+SD)(mg)		
	Replicate 1	Replicate 2	<i>P</i>	Replicate 1	Replicate 2	<i>P</i>
Successful ^a	14.9 ± 4.19	14.6 ± 4.24	< .05	865 ± 36.1	864 ± 49.8	< .05
Unsuccessful.....	18.1 ± 4.56	17.8 ± 5.94		837 ± 39.8	835 ± 48.2	

^a Successful larvae are significantly ($P < .005$; *t*-test) heavier and pupate significantly earlier (*t*-test) than unsuccessful larvae.

chambers or the arena during the 2-day dispersarium phase. However, this does not completely account for the increased mean time to pupation, since this is somewhat greater than 3 days in both replicates.

CHAMBER DEFENSE BY IMMOBILIZED LARVAE,
PHARATE PUPAE, AND PUPAE

Since all the foregoing experiments indicated that the ability of occupants to exclude challengers was a behavioral phenomenon and required occupant mobility, an experiment in which the occupants were immobilized to eliminate all behavior but were otherwise normal was devised.

Immobilization of larvae was accomplished by covering each of the spiracles with a spot of molten beeswax. The resulting anoxia caused all movement to cease and was similar to the anesthetic state induced by submerging the larvae in water, as for surgery. Such waxed larvae usually lived for at least 48 h, though some localized tissue death occasionally occurred. In such cases, a fresh larva was substituted. The controls were similar except that the wax spots were placed adjacent to the spiracles and the larvae were therefore not immobilized. Both immobilized and control larvae were marked with silver paint.

Each of the two replicates consisted of placing 26 immobilized or control occupants, one each, into the chambers of the

dispersarium. After 24 h, 26 challengers fresh from the same mass culture (age 11–12 mo; weight 650–750 mg) were added to the arena of the dispersarium and allowed to disperse for 24 h. Chamber occupancy was noted, as before. Immobilized larvae which became active, were injured, cannibalized, or died of their own accord, were replaced during the experiment.

In two additional experiments (two replicates each), pharate pupae or pupae from the same culture were similarly used as occupants. All four experiments were analyzed together as three treatments (immobilized, pharate, pupae) and a control. Analysis of variance was carried out on the dependent variables (1) total in chambers, (2) challengers in chambers, (3) occupants sharing, (4) challengers sharing, and (5) successful occupants. The increased ability to exclude intruders would have the effect of decreasing all of these except successful occupants, which would increase. The treatment had a significant effect ($P < .05$, table 4) in the expected direction on three variables: total in chambers, occupants sharing chambers, and challengers sharing chambers. Since there was no significant effect of challenge time on any of the variables, and since the relative rank of the dependent variables was usually constant throughout the experimental period, the mean values

TABLE 4
 MEAN VALUES OF FIVE DEPENDENT VARIABLES (criteria of chamber defense)
 IN RELATION TO FOUR TREATMENTS (occupant type)

TREATMENT (Occupant Type)	DEPENDENT VARIABLES				
	Total in Chambers	Challengers in Chambers	Occupants Sharing	Challengers Sharing	Successful Occupants
Immobilized larvae.....	51.1	25.2	20.2	25.2	5.8
Pupae.....	46.5	20.5	17.8	20.5	8.4
Pharate pupae.....	41.9	16.4	14.3	13.2	10.8
Control (active larvae)....	35.8	17.7	6.8	9.0	10.9
<i>P</i> -value (factor: treatment)	.05	NS	.05	.05	NS
<i>P</i> -value (factor: challenge time).....	NS	NS	NS	NS	NS

NOTE.—Last two rows give the *P*-values for the effect of the treatments and challenge time on each dependent variable.

over 24 h for each of the four treatments are listed in table 4.

It is apparent (table 4) that ability to exclude active larvae from the chamber is least for completely immobilized larvae, greater for pupae, greater still for pharate pupae, and greatest (often by a wide margin) for active larvae (controls). For example, total in chambers for immobile occupants was almost always near the maximum of 52, while for controls it ranged from 38 to 33. The other variables showed similar patterns, with pupae and pharate pupae ranking between the other two groups. Even though they are unable to walk about, pharate pupae have some ability to exclude intruders by thrashing violently from side to side. Pupae can flick the abdomen, bringing the gin traps into play.

Since the treatment occupants, once placed into a chamber, were unable to leave, the ideal control should consist of 26 active larvae able to defend, but not leave, their chambers. The actual control larvae move freely among the chambers and arena and are thus an imperfect control. This shortcoming is partly but not completely overcome by considering only the occupants actually in the chambers (this value fluctuates).

An additional χ^2 test was carried out on each replicate of the four experiments comparing the observed number of chambers containing more than one larva of either type with the number expected on the basis of random assortment of larvae into chambers (Tschinkel and Van Belle 1976). During the first 6 h, none of the treatments were significantly different from the expected, but from 20 to 25 h the experiments with immobilized larvae showed more chambers with multiple occupancy than expected by chance ($P = .05$), while the controls showed significantly fewer such chambers (P marginal at about .05). The immobilized larvae are unable to exclude intruders, an ability which the control larvae possess. Neither of the other two treatments was significantly different from random with respect to multiple occupancy.

One of the most dramatic results of using immobilized occupants was the enormous rate at which these were cannibalized by the challengers. In the first of two replicates, there were almost two complete changes of larvae over a 24-h period. That is, 49 larvae had been killed by this time. The total was about 38 in the second replicate. In several cases, challengers were observed to enter a

chamber and almost immediately began cannibalizing the immobilized occupant. The normal rate of cannibalism of active larvae in a dispersarium experiment is usually less than 1 in 100. It seems apparent that the primary barrier between a larva and cannibalistic annihilation is active defense or avoidance. The magnitude of this effect was unexpected.

DISCUSSION

Evidence presented here indicates that the overdispersal of mature larvae of *Zophobas rugipes* among pupal chambers results from primarily behavioral exclusion of intruders by active defense. When the occupants are not able to behave (immobilized), the challengers are not only able to enter the chambers but, in essence, disperse in relation to one another as though the occupants were not there. Thus at first the dispersal of the challengers in relation to one another is random, but after 24 h each chamber usually contains only one challenger. If the immobilized occupants are ignored, these results are essentially identical with the dispersal of only 26 larvae, rather than 52—i.e., all larvae are in chambers and there is one per chamber.

The motivation to defend increases rapidly with isolation time in the chamber but is not affected by the age or weight of the pupation-ready larvae. Larvae still in the growth phase show a strong relation among weight, success of chamber defense, and tendency to pupate, which indicates that the motivation appears during the growth and maturation of the larvae but does not change radically after maturation.

The ultimate outcome of these behavioral and developmental interactions is that pupation-ready larvae disperse until they find an undisturbed site, whereupon they dig a chamber and

pupate. The evolutionary cause of this overdispersal phenomenon as well as the inhibition of pupation by crowding is probably the strong cannibalistic tendency of the larvae (Tschinkel and Van Belle 1976). Larvae which pupate in the vicinity of active larvae are strongly counterselected by cannibalism, while those dispersing away from crowded areas are favored. From the point of view of the cannibal, conspecific larvae are an unusually rich source of food and could give the cannibal certain selective advantages, as it does in *Tribolium* where egg-eating larvae are more fecund as adults (Ho and Dawson 1966). Cannibalism could also remove competitors. Possible advantages of cannibalism have not yet been tested in *Z. rugipes*. The voraciousness of the cannibals and the vulnerability of the immobilized larvae were impressive and emphasize the danger presented by this habit to the vulnerable life stages.

Pupal chamber defense is perhaps most similar to the territoriality of vertebrates and insects (Brown 1975). In both territorial and chamber defense, the occupant defends its space against conspecifics, resulting in an overdispersed distribution of individuals. Motivation is intrinsic to the territory holder and important to success, just as it is in chamber defense. A more aggressive individual can displace a territory holder or a chamber occupant. Competition for territory and chambers is intensified by increasing population density, and those individuals unable to compete for favorable sites are subject to higher mortality. Under conditions of high population density, territory can become the limiting resource for which animals compete (Brown 1975), and it seems possible that competition for pupation sites could play a role in population limitation.

LITERATURE CITED

- BROWN, J. L. 1975. The evolution of animal behavior. Norton, New York.
- GHEENT, A. W. 1966. Studies of behavior of the *Tribolium* flour beetles. II. Distribution, in depth of *T. castaneum* and *T. confusum* in fractionable shell vials. *Ecology* 47:355-367.
- HO, R. K., and P. S. DAWSON. 1966. Egg cannibalism by *Tribolium* larvae. *Ecology* 47:318-321.
- KENNEDY, J. S. 1956. Phase transformation in locust biology. *Biol. Rev.* 31:349-370.
- KING, C. E., and P. S. DAWSON. 1973. Habitat selection by flour beetles in complex environments. *Physiol. Zool.* 46:297-309.
- LEES, A. D. 1961. Clonal polymorphism in aphids. Pages 69-79 in J. S. KENNEDY, ed., *Insect polymorphism*. Symposium of Roy. Entomol. Soc. London. Vol. 1.
- LEONARD, D. E. 1970. Intrinsic factors causing qualitative changes in populations of *Porthetria dispar* (Lepidoptera: Lymantriidae). *Can. Entomol.* 102:239-249.
- MARLE, G. 1951. Observations on the dispersal of the fruit tree red spider mite *Metatetranychus ulmi* (Koch). Pages 155-159 in E. Malling Research Station annual report for 1950.
- NAYLOR, A. F. 1965. Dispersal responses of female flour beetles, *Tribolium confusum*, to the presence of larvae. *Ecology* 46:341-343.
- TSCHINKEL, W. R., and C. D. WILLSON. 1971. Inhibition of pupation due to crowding in some tenebrionid beetles. *J. Exp. Zool.* 176: 137-146.
- TSCHINKEL, W. R., and G. VAN BELLE. 1976. Dispersal of larvae of the tenebrionid beetle, *Zophobas rugipes*, in relation to weight and crowding. *Ecology* 57:161-168.