ABSTRACT

THE RELATIONSHIP BETWEEN BIOLOGICAL CONDITIONING AND ANIMAL-ANIMAL INTERACTION IN THE SPATIAL DISTRIBUTION OF GALLERIA MELLONELLA (LEPIDOPTERA) LARVAE

By

Peter Edward Hogan

The studies in this dissertation deal with the preference behavior of individual Galleria mellonella (L.) larvae for homotypically conditioned food, its relationship to spatial distribution, and the effects of degree of conditioning, development (age), and the presence of conspecifics on these preferences. A mechanism is postulated for the interaction of these variables with larval activity rhythms in determining spatial patterns. Also presented is an analytical technique for the analysis of multiple samples taken from the same individuals over time.

In Chapter I is presented a review of the literature on spatial distribution as it specifically relates to invertebrates with an emphasis on insects and the role of biological conditioning in spatial distribution. The effects of animal-animal interactions on preferences for conditioned food are discussed. The studies in this review are population studies in which individual behavior, in respect to homotypic conditioning and animal-animal interaction, has not been thoroughly investigated.

Chapter II presents many life history and behavioral considerations directly pertaining to Galleria mellonella larvae, the experimental organism for these studies.

Chapter III presents the statistical procedures and the underlying assumptions for multivariate analysis of longitudinal data. Hotelling's One and Two Sample T^2 Statistics for Multivariate Analysis are outlined, illustrating a procedure for treating time as a multidimensional variate. Emphasized are procedures for testing the assumptions of homogeneous variances and non-serially correlated data. The major objective is to arm behavioral biologists with the necessary analytical techniques enabling logical decisions about tests of significance on repeated individual measurements over time.

Chapter IV tests the hypothesis that preference of individual Galleria larvae for homotypically conditioned food is a function of the degree of conditioning and age of the larvae. Larvae were given a two-choice situation in which one food lump was variously conditioned and the other was non-conditioned. Individual preference was recorded every 12 hours for the total developmental period. Depending on the degree of conditioning, larvae are attracted to conditioned food with a threshold effect being indicated at high levels of conditioning. Older larvae demonstrate stronger, initial, attraction to conditioning than do young larvae, although there is some confounding with early experience factors, but initial preference is not a good indicator for long-term preference. There are indications that age differences in preference may be related to differential activity. Adult Galleria, on the other hand, avoid highly conditioned food, avoidance being

measured by oviposition preferences. The consequences of this behavior are discussed in relation to larval density.

Chapter V treats the hypothesis that individual larval preferences for conditioned food are a function of the presence of conspecifics. A resident larva (always an older larva) in a conditioned food lump has no effect on individual preference for that food lump. However, if two larvae encounter each other in the open, that is when neither larva is in a food lump, both preferences for conditioned food are lowered, as measured by comparisons with an isolate situation. The preference of the younger larva is most affected initially but soon increases, possibly as a function of an interaction with the older larva. A hypothesis was proposed and tested that the behavior of the younger larva is a function of the initial encounter between the two larvae, extra conditioning performed by the older larva on the already conditioned food lump, and a second encounter later in development. The effect of the initial encounter is supported by the data, but the effects of the other two variables remain inconclusive. Future tests are proposed.

In Chapter VI are summarized the results of these studies. Individual behavior and distribution of Galleria larvae is a function of the degree of homotypic conditioning, age of the individual larva, presence and age of a conspecific, location of the conspecific, and possibly activity differences between larvae.

THE RELATIONSHIP BETWEEN BIOLOGICAL CONDITIONING AND ANIMAL-ANIMAL INTERACTION IN THE SPATIAL DISTRIBUTION OF GALLERIA MELLONELLA (LEPIDOPTERA) LARVAE

By

Peter Edward Hogan

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Zoology

1975

I dedicate this dissertation to my wife, Peggy, whose incalculable patience, understanding, and help have made all phases of this study possible.

ACKNOWLEDGMENTS

I wish to express my appreciation to Dr. Martin Balaban, my advisor, for his help and encouragement throughout my graduate career and for his critical evaluation of my research and this dissertation. I am also grateful to the members of my guidance committee including Dr. John A. King, Dr. James H. Asher, and Dr. Stanley C. Ratner for their constructive comments during this research.

Special thanks go to Dr. James H. Asher for the time spent helping me with the mathematical analyses in this dissertation and to Lincoln Gray for his invaluable help in writing the computer programs used in the analyses. Without their help this dissertation would not have been possible.

This research was supported in part by NIH Animal Behavior Training Grant #5 TO1 GMO1751-05 BHS and by teaching assistantships provided by the Department of Zoology.

TABLE OF CONTENTS

LIST OF TABLES

Table | Page

Table | Page

Table Page

Table | Page

LIST OF FIGURES

Figure Page

CHAPTER I

BIOLOGICAL CONDITIONING AND SPATIAL DISTRIBUTION

Introduction

Biological conditioning has been defined (Allee 1934) as ". . . changes produced in the medium by organisms living therein and the effects of such changes upon other organisms . . . ." Changes in the medium may be chemical or physical and either of homotypic or heterotypic origin. Homotypic conditioning is produced by a conspecific, whereas conditioning emanating from other than a conspecific is referred to as heterotypic. As organisms inhabit their environment they modify it, often to such an extent that whole populations are affected. With invertebrates, notably Lepidoptera, a few studies have related biological conditioning to spatial patterns, although most of the literature treats conditioning in relation to its physiological effects upon other organisms and populations. Since biological conditioning was considered a population phenomenon, these studies looked at whole populations, making no attempt to ask questions about how biological conditioning affects individual behavior.

The research for this dissertation deals with an analysis of spatial distribution (aggregation and/or dispersal) in the wax moth larvae, Galleria mellonella (Lepidoptera), when maintained in the laboratory. In a simplistic sense, aggregation may be viewed as the

result of those factors that "pull" organisms together, and dispersal as the result of those factors that "push" them apart. Spatial distribution (dispersion) would be the result of the interactions between these "pulls" and "pushes." Relationships between biological conditioning, animal interactions, and development (age) are examined. The basic approach is to analyze spatial distribution by isolating the critical variables in simple systems of individuals or small groups. Concomitant with this biological approach is the development of an analytical model for spatial distribution.

Galleria mellonella was chosen for these studies because of its suitability for behavioral, distributional, and populational experimentation, and because the larvae produce a pronounced physical (and possibly chemical) conditioning of their environment.

Galleria larvae are vagile, asocial, asexual, short-lived and herbivorous. Naylor (1959) pointed out that these features allow the analysis of behavioral and spatial relationships. Vagility is the ability to actively move from place to place. Asociality is here defined as the ability of an individual to survive and/or reproduce in the absence of structured groups. Galleria adults are extremely fecund, enabling generation of large sample sizes for experimentation. The implication of being short-lived is that Galleria larvae must quickly respond to cues. Herbivorous species usually find food and shelter in a relatively fixed location, and Galleria larvae are not normally found ranging over vast expanses of territory for their life needs. The adults lack mouth parts, do not feed, and are not usually found in close association with the larvae.

A consequence of the preceding traits is that the behavior of Galleria is a relatively "simple" system for elucidating the critical variables relating to spatial distribution. Several variables present in other systems, such as sexual relationships and adult-larval interactions, are not of prime importance.

The following general hypotheses were examined in this dissertation:

1. The spatial distribution of Galleria larvae is a function of their preferences for homotypic conditioning.
2. Preferences for biological conditioning depend upon the degree of conditioning and the age of the responding larvae.
3. Animal-animal interactions (presence of conspecifics) alter preferences for conditioning, and the degree to which preferences are altered is a function of age and prior residence in the conditioned situation.

Literature Review

Spatial Distribution

Dispersion is the spatial structure of a population, as opposed to dispersal which is the dynamic process involved (Surtees 1963a, 1964c). Lack's (1954) definition is more rigorous. He defines dispersion as a non-random type of distribution, with dispersal being the movement of young animals from their place of birth. Dispersal is normally thought to consist of (1) organisms leaving their natal site, (2) crossing some sort of barrier, and (3) settling and breeding in a new place. These criteria have strong genetic implications. However, for the purposes of my studies, dispersal is being defined as the

process by which an organism or group of organisms move away from some original site, whether or not breeding occurs in the new locality. In this context dispersal is the opposite of aggregation, and is merely one side of a broader concept, dispersion. Wynne-Edwards (1962) defines dispersion as the placement of individuals and groups of individuals within the habitats they occupy, and the processes by which this is brought about. A general definition, such as this, encompasses other than non-random groupings.

Dispersion, contagious distributions, aggregations, groups, bunches, clusters, or whatever label one wishes to give such phenomenon, are of widespread occurrence in nature, and are found in varying degrees of complexity throughout the animal phyla. They range from chance assemblages to the highly organized social insects and man. Natural aggregations may result from the response of individuals to the presence of conspecifics or from the independent response of each individual to an external stimulus (Bowen 1931), the former often being called social and the latter asocial. Allee (1927), however, noted that once established, aggregations may last for extended periods of time merely due to a lack of disruptive stimuli; whereas dispersal may be the result of a lack of cohesive stimuli. No social instinct need be evoked to account for the formation or maintenance of aggregations. Wynne-Edwards (1962), on the other hand, states that the maintenance of the spatial organization of animal populations can be attributed to behavioral interactions among its members. An implication of this statement is that whatever the causative factors of aggregation are,

individuals would exhibit different distributions if other conspecifics were absent.

The critical variables being considered in my studies are biological conditioning and animal-animal interaction. Other causative factors in spatial patterns are frequently encountered in the literature, such as the role of the sense organs, food, and various environmental cues. In addition, the mobility of organisms is important and this may depend on individual variables such as sexual and genetic differences. These factors must be either controlled or considered as variables. It is not the intent of this review to discuss each of these at length. However, since such variables can affect preferences for biological conditioning as well as animal-animal interactions, and since they form the framework for much of the spatial distribution literature, they will be briefly discussed.

Sense Organs

Sense organs serve as the passageway by which all sensations from the environment reach the central nervous system and elicit some kind of response. Auditory, visual, olfactory, tactual, and electrical signals may all function in dispersionary as well as social behavior. (See Wynne-Edwards 1962 for a review of this topic.)

Two basic approaches might be used to study the effects of biological conditioning on spatial distribution. One is to find out what constitutes conditioning and which senses are employed in responding to it. The second approach is to demonstrate a response to conditioning, regardless of the senses employed or the characteristics of conditioning, and begin asking questions about the results, function,

and dynamics of the responses. I have opted for the latter approach in my studies, although the first approach must eventually be studied. To study the relationship between biological conditioning and spatial distribution it is not necessary to know what conditioning consists of or what senses are used to respond. It is, however, necessary to demonstrate that conditioning is occurring, that it emanates from the organism in question, and that the organism responds to it. The degree of conditioning can be controlled in terms of time spent conditioning and age of the organism doing the conditioning.

Food and Spatial Distribution

Most animals, through their power of movement, play a part in determining their own population densities. They are able to concentrate at places where food and other resources are plentiful and to avoid unfavorable situations. According to Wynne-Edwards (1962), food is always the critical or limiting resource which dictates how high a population density can be supported in any particular habitat. Regardless of food supply, however, other variables such as animal-animal interactions and biological conditioning play a major role in distribution.

Wynne-Edwards (1962), using some of Jesperson's (1924) data noted that a high correlation exists between plankton density and the density of pelagic birds. He concluded that the birds disperse themselves on the basis of available food supply.

Territoriality, a form of dispersion, in birds may be food limited. Howard (1920) found that territorial size may be reduced in the presence of abundant food, and may increase in size when food

becomes limited. The relationship between food, dispersion, and numbers in a population was discussed by Lack (1954) who concluded that many bird species are limited in numbers, and in resultant distribution, by their food supply.

Similar data is available for other species as well. Calhoun (1948, 1952) with rats, and Brown (1953) and Strecker (1954) with house mice, have demonstrated that location of food may be a factor in influencing spatial distribution under semi-natural conditions. Calhoun's (1950) study also shows that population size and competition are important in determining spatial distribution. However, the results of such experiments indicate that being near food enables elimination of other individuals from the area. This is an indication that animal-animal interaction, not food supply, is the proximal cause for their distribution.

Food has often been shown to be a variable in the spatial distribution of invertebrates. Loschiavo (1952) has shown that Tribolium confusum exhibit strong food preferences which in turn affect its distribution. In fact, flour is not the preferred medium of the flour beetle, if a more nutritional medium is available. Galleria mellonella larvae also utilize foods differentially as well as exhibiting food preferences (Chase 1921; Milum and Geuther 1935; Whitcomb 1946; Beck 1960).

Crowding, often related to food, may affect an organism's physiology as well as its distribution. In a study on crowding of the larvae of the meal moth, Ephestia kuhniella, Smith (1969) found that larvae infesting food supplies in an uncrowded state grow faster and

larger than those in crowded substrates. This is rather a common observation among other species as well, and has been observed in our laboratory with Galleria larvae. However, Smith did not determine if stunted growth was due to crowding, food limitation, or excessive conditioning of the medium. Smith also observed that less crowded cultures of Ephestia reproduce faster and probably more plentifully. Rose (1959) discovered similar results in guppies, as have Sang (1949) in Drosophila melanogaster, Park (1938) in Tribolium confusum, Brown (1946) in trout fry, and Rose (1960) in tadpoles. Smith postulated that this faster and more plentiful reproduction maximized population size in the next generation, ensuring maximal use of a food supply. However, none of these researchers made any attempt to separate the effects of crowding and food supply from the effects of biological conditioning in crowded cultures.

Smith (1969) also noted a behavioral response of Ephestia adults in crowded cultures, the adults being more active than isolates. Such activity may promote the colonization of suitable surrounding food supplies as they become available and as the old supply becomes crowded.

A behavioral response of organisms to some aspect of their food supply may affect their distribution. Butterfly larvae of Mechantis isthmia avoid the trichomes on their Solanum plant host by spinning a network of silk over the tops of the spines (Rathcke and Poole 1975). The larvae can then crawl over the tops of the spines on their "silken scaffolding" and safely feed on the unprotected edges of the leaves. Most larvae of this genus are solitary feeders, but

larvae of Mechantis isthmia generally feed in small groups of from four to six individuals per leaf, spinning and sharing the webbing together.

Rathcke and Poole (1975) advanced the hypothesis that such a silken bridgework is energetically feasible only if several larvae pool their resources and share the benefits. The ability to spin silk is widespread among the Lepidopterans, as well as in other orders. Most larvae spin silk to secure themselves while molting or to attach or protect their pupa. Therefore, evolution of a feeding web would entail only an elaboration of an already present ability. However, the increased silk production could necessitate a change in the social behavior from solitary to gregarious. Such an hypothesis might also apply to Galleria larvae if Paddock's hypothesis (1913) that silk tunnels function to hold the damage combs together after feeding is tenable.

There are also situations in which food is not limited and the distribution of a population of organisms is a function of how they behaviorally exploit a preferred food supply. Aphids feed on the phloem cells of plants, imbibing soluble nutrients such as sugars and amino acids. Observations by Way and Cammell (1970) indicated that aphids normally aggregate in groups of about twenty, and that a relationship might exist between these aggregations and food utilization. They discovered that aggregates of aphids act as "sinks" diverting nutrients from distant plant parts, and that isolates could not as effectively feed. However, as these aggregates enlarged, population growth was halted as a result of decreased natality. Therefore, aphids

with spaced out populations, or in small aggregations, do little harm to a plant and maintain relatively stable populations. Way and Cammell further noted that different species exhibited behavioral differences in exploitation of host plants, with a resultant difference in aggregation behavior. Those species normally exploiting a host plant piecemeal remained closely aggregated, i.e., the cabbage and bean aphids, whereas those species normally doing little harm to a host plant remain spaced out in tiny aggregations, i.e., peach and potato aphids.

There are innumerable examples of the effects of food on spatial patterns, and food is probably the ultimate factor in any long term dispersionary study. The main reason for discussing it, other than elucidating it as an important variable, is that many studies in spatial distribution failed to account for limited food supplies. Several studies in this review failed to do so, and, as such, require cautious interpretation. For example, the results of Surtee's studies (see "Environmental Factors in Spatial Distribution") are interpretable on the basis of upward movement away from used up food supplies, rather than on the basis of Surtee's hypothesis of upward dispersal under high density as a kinesis. Similar problems arise in Park's work with Tribolium, and Park admitted that the food limitation hypothesis needs testing before his physiological and distributional data can be verified. However, all of my studies described in this dissertation controlled for limited food source as a dispersionary agent.

Environmental Factors in Spatial Distribution

It is well documented that organisms respond to environmental cues, such as temperature, light, and various structural parameters of the environment, and that such responses, often associated with physiological tolerances, may affect spatial patterns. Selected examples from vertebrates and invertebrates illustrate this point.

Studies in habitat selection (Harris 1952; Wecker 1963; Fitch 1974) have demonstrated that mice prefer certain habitats, which may be related to environmental, genetic, and early experience variables. Habitat characteristics, such as fallen trees and holes in the ground, have been shown to affect the spatial distribution of small mammals. Brand (1955, from Terman 1961), using Peromyscus leucopus, showed a direct seasonal relationship between population distribution and tree density, density of fallen trees, and degree of slope of fallen trees. The significant factors in this relationship are probably potential nest sites and food. The number of rats in city blocks was temporarily decreased by removing harborage sites (Orgain and Schein 1953), and Davis (1958) changed the spatial distribution in laboratory populations of house mice by introducing baffles and additional nest sites.

Moisture, ground depressions, hard textured logs, plant cover, and high relative humidity all positively influence the movements of the salamander, Plethodon cinereus (Heatwole 1962). Most of these variables are directly related to the salamander's physiological limits. Varying spatial patterns in relation to environmental stimuli and physiological tolerances have also been documented in the ant Formica (Talbot 1934), termites Reticulitermes hesperus and

R. tibialis (Williams 1934), grasshopper Melanoplus (Parker 1930), salamander Plethodon (Shelford 1913), and wireworm populations (Salt and Hollick 1946; Thorpe et al. 1946). In some instances such varying distributions are seasonally correlated.

Often spatial distribution is not so much a result of environmental factors as it is a result of an organism's mobility limits. For example, Bovbjerg (1952) investigated the aggregation behavior of the aquatic snail Campeloma decisum and found that these aggregations were a result of an upstream dispersal, probably a rheotaxis, coupled with an inability to traverse even slight blockages of the streams.

Abiotic variables, such as light, temperature, and humidity, are the most commonly investigated variables in spatial distribution. In the spruce budworm (Choristoneura fumiferana), for example, Wellington (1948) demonstrated that young stages aggregated as a result of being photopositive. Older larvae exhibit a reversal in this orientation. Phototaxis is also the predominant factor in groupings of the sea urchins Arbacia punctulata and Lythechinus variegatus (Sharp and Gray 1962). Arbacia is negatively phototactic. Lythechinus is positively phototactic to artificial light and negatively phototactic to sunlight. Lythechinus also exhibits an interesting "heaping" behavior as a response to light which consists of picking up shells and seaweed and covering its body with them.

There is considerable literature on the spatial patterns in isopods in relation to abiotic factors. Warburg (1964) compared several isopod species, finding aggregation differences in relation to light, temperature, and humidity. Previously Allee (1926) and

Cole (1946) had shown that temperature and dryness affect the "bunching" phenomenon in isopods. Warburg, however, tested several species and discovered species as well as mechanism differences in response. In the grain beetle (Sitophilus granarius), vertical distribution depends upon temperature, humidity, and density of organisms (Surtees 1963a). At low and high temperatures the number of weevils at the surface increases as a function of density. Increased moisture decreases surface aggregations. In a study on the saw-toothed grain beetle (Oryzaephilus surinamensis), Surtees (1963a) discovered the same kind of relationship between density and moisture, but species differences were observed. Species comparisons of vertical aggregation behavior in relation to moisture, temperature, and density were therefore performed by Surtees (1964e). The general conclusion from these studies is that accumulation of adults occurs under those conditions where kinesis becomes minimal. Reduction of individual kinesis levels has a secondary effect within groups of adults in that it depresses mutual disturbance which would otherwise elicit dispersal. Accumulation within the physical conditions of temperature and moisture is therefore affected by changes in orthokinesis and klinokinesis. In all species examined, specific rates of turning and levels of speed under various conditions of temperature, moisture, and density were found. Aggregation would be expected in regions of lowest velocity and fewest turns. These conditions prevail under the proper conditions of temperature and humidity. Increasing density has the opposing effect of dispersing the weevils away from such aggregations. However, the effects of biological conditioning and limited food

reserves were not controlled in these experiments and both are viable alternative hypotheses to Surtees' explanation for dispersal.

Surtees (1964f) further discovered that aggregation behavior is affected by the beetle's early experience with humidity differences. Beetles reared at 70% R.H. did not accumulate in damp grain, whereas those reared at 40% R.H. for 14 days did. Humidity-conditioned adults moved slowly in damp grain, unconditioned adults moved rapidly. These results add further support to his hypothesis that accumulation in one area must be seen as the joint result of a reduction in individual movement and a consequent depression of intra-group disturbance, which would otherwise elicit dispersal. A similar result was found by Graham (1958) with Tribolium confusum and T. castaneum. Both species exhibit temperature preferences which affect their dispersal. Early experience, however, alters these preferences.

Dispersion behavior is often interpreted in light of the variable being examined, when in fact some other, possibly uncontrolled, variable is the actual mechanism. The woodlice literature is a case in point. Woodlice live in places of high moisture content, i.e., beneath bark, under rocks and fallen trees, or beneath leaves, and humidity had been accepted as the controlling variable in their habitat choice and degree of activity. However, since crevices are dark, as well as damp, phototactic and hygrokinetic behaviors were probably linked (Edney 1954). It had also been assumed but never tested, by Allee (1926), that woodlice react to contact stimuli, since crevices are confining and when crevices are not available they climb on each other and form aggregations. Friedlander (1964) attempted to

sort out the responses of the woodlice Oniscus ascellus, Porcellio scabes, and Armadillidium vulgare and how they relate to aggregation behavior. His particular interest was with thigmokinetic behavior, or the response to contact. The results indicate that although the effects of thigmokinetic reactions are similar to humidity reactions, they are distinct from them. Such responses also varied with species and degree of humidity, being most marked at low humidity. The thigmokinetic responses depend upon area and roughness of the contacted surface, and increases in either results in reduced locomotion.

It would appear that woodlice are drawn to crevices by humidity responses, and that if the crevice provides the proper thigmokinetic stimuli, movement is slowed and aggregations form. However, Friedlander did not perform experiments on thigmokinetic behavior as a result of touching other individuals so attracted to a crevice. It is reasonable to assume that as the number of individuals increases in a crevice, thigmokinetic responses to these individuals may override the tendency to slower movement and lead to dispersal. This is very much what happens in grain beetle populations, as seen earlier.

It is obvious that as environmental factors vary, so does spatial distribution. In all of my experiments, however, light, temperature, and humidity were held constant in order not to be involved in any spatial distribution results with Galleria populations. This was necessary for several reasons, one of which is that there are indications that abiotic variables affect biological conditioning. For example, low temperatures decrease spinning activity and spinning activity may be the main component of conditioning in Galleria larvae.

Sex and Genetic Factors in Spatial Distribution

The mobility of an organism has important consequences in relation to the ability to aggregate or disperse. Mobility may depend upon the individual variables of sex and genetics. McDonald (1968), using Tribolium confusum, found that males more rapidly disperse than females and that there are strain differences in mobility. Strain differences may be due to differential response to repellent substances released by the organisms or to avoiding other adults. This was not tested. However, he did find that the mobility of males is lessened in the presence of a female. This supports Naylor's (1959) finding that male Tribolium confusum are attracted to females and exhibit a different distribution pattern than if females are absent from the population.

Since genetic differences in mobility and dispersal do exist, it would be helpful to measure the amount of genetic variation populations contain for such traits (or at least control them in experimentation) and to explore the relationship between mobility and other fitness components such as fertility and fecundity. In Tribolium, certain behavioral traits are easily studied and modifiable by artificial selection. Dawson (1964) found that individuals of highly inbred lines of Tribolium confusum spend a greater percent of time on the surface of the flour medium than the normal wild type. Sokoloff (1966) extended this finding to mutant strains and demonstrated that the response was maintained independently of population density, in culture containers. The general activity levels of wild type and certain body color mutants were compared by McDonald and Fitting (1965)

who found the latter to be more active. Using Tribolium confusum and T. castaneum, Lerner and Inouze (1968) were able to achieve rapid response to selection for speed of running hierarchical T-mazes.

Artificial selection has demonstrated that mobility may be altered, but the exact mechanism is as yet unclear. It is possible that what is being selected for is response to some cue rather than increased mobility. Ogden (1969) experimented with some olfactory cues which mediate dispersal behavior in Tribolium. He attempted (1970b) to determine dispersal behavior, defined as emigratory movement by walking in a laboratory apparatus. The apparatus utilized was that originally designed by Prus (1963), consisting of two shell vials connected with a U-tube through which a thread was strung. The apparatus was so designed as to allow only one-way movement through the tube. Ogden tested fourth generation beetles as to their rate of dispersal from homotypically conditioned medium and fresh flour. In T. castaneum the rate of dispersal increased with selection, as compared to dispersal from fresh medium, while it was depressed in T. confusum. Selection, therefore, served only to modulate the normal response of each species. (T. confusum normally aggregates in homotypically or heterotypically conditioned flour, whereas T. castaneum is normally repelled by both.) Ogden's results clearly indicate a genetic component to dispersal behavior. However, it is not clear whether the genetic component relates to increased mobility or increased preference for preferred medium.

The extent of selection, such as Ogden performed, in natural populations and its consequences to population dynamics is largely

unknown. However, some work has been done in this area. Wellington (1957), using a behavioral test for the ability of the tent caterpillar (Malucosoma pluviale) to move towards a light source when isolated, found two distinct behavioral populations. Type I larvae were capable of independent, directed movements as isolates and in groups. Type II larvae consisted of larvae that were somewhat active if other larvae were present, larvae that were almost always disoriented, and larvae which hardly moved at all. The consequences of these behaviors to spatial distribution will be discussed later. The relevant point at this time is that these larval types have a genetic component related to mobility differences. Since such differences were also found in the wild, Wellington's results are not a laboratory artifact.

Since it was not my intention to investigate the genetic factors in the spatial distribution of Galleria larvae, sex and genetics were controlled as closely as possible. For purposes of my experiments, I attempted to keep genetic variability, relating to spatial distribution, constant. As discussed in Chapter II, new stocks of larvae were periodically introduced into the breeding colony and behavioral tests utilized as indicators of whether response to biological conditioning fluctuates. The results of these tests indicate a constant response over time. These results demonstrate that variability in response differed over time, but the average behavioral response remained constant over the period tested.

Sexual differences were of no concern in my experiments with Galleria. As near as anyone has been able to determine, Galleria larvae exhibit no sexual dimorphoism. For purposes of the

experiments in this dissertation, therefore, Galleria larvae are assumed to be sexually undifferentiated. However, there may be a behavioral dimorphism relating to sexual differences which may account for a component of the observed changing variance over time. This was not investigated.

Density Factors in Spatial Distribution

One of the variables investigated in this dissertation is the effect conspecifics have on one another in terms of spatial distribution and if the results of such animal-animal interactions are altered as age, number of organisms, and degree of biological conditioning are manipulated. The discussion at this point will consider density dependent factors commonly associated with population spatial patterns. Later we will discuss similar considerations in terms of biological conditioning. The density related literature in invertebrates, particularly insects, is of main concern to my studies. However, relevant hypotheses derived with rodents will be briefly touched upon.

Stickle (1946) and Calhoun and Webb (1953), using small mammals, have shown that stability of spatial distribution is related to the number of animals in an area. Animals in surrounding areas tend to move to vacated areas following removal of the residents. Introduction of animals into already populated areas causes them to disperse (Calhoun 1948) or to so disrupt the population that a temporary decline in numbers occurs (Davis and Christian 1956).

Christian (1970) states that there may be a direct relationship between the degree of aggressiveness and the magnitude of dispersal

with increased density and the associated conspecific contacts. As numbers increase, the level of aggressive interaction may be increased, and with it the tendency to disperse. Christian was referring to mammal populations. However, these statements are generally applicable to any population of organisms if the term aggression is dropped, until we have a better understanding of what it entails, and simply refer to animal-animal interactions. Therefore, there is a direct relationship between the degree of animal-animal interaction and the magnitude of dispersal with increasing density. At one end of the continuum are low density aggregations with few interactions, and at the other end is high density dispersal with many interactions. These statements are particularly relevant in the so-called "asocial" organisms which have not yet evolved sophisticated mechanisms or divisions of labor to unify large masses of animals and in which there is no obvious benefit derived from large aggregations.

Perhaps the best known works dealing with cyclical fluctuation of populations are those of Christian (1950), Chitty (1955), and Krebs (1966) and Krebs et al. (1969) dealing with rodent populations. (Although cursory to the main thrust of this review, these authors are briefly mentioned because their theories relate to animal numbers and spatial distribution and are relevant to some other papers to be discussed, namely Wellington (1957, 1960) and Long (1953).) Myers and Krebs (1974) have written an excellent review of this area. Christian hypothesized that high densities of organisms produce "stress," i.e., disorders of the endocrine system, and thus precipitate a decline in the population, whereas Chitty felt that the effects of

high density were to be found in the offspring, not in the exposed generation. Chitty further hypothesized that high density stress is genetically selective and that social interactions within the population would change during the population cycle. Krebs tested several of Chitty's hypotheses, as well as his own ideas about dispersal, and developed a model of rodent population cycles. The model postulates that two genotypes are present in rodent populations; one adapted to survival under crowded conditions but having a low reproductive rate, and the other being reproductively superior but intolerant of high densities. As population size increases, the density-intolerant individuals leave.

Krebs' hypothesis may be applicable to spatial distribution in insects. Wellington's (1957, 1960) work with tent caterpillars demonstrates the presence of two genetically distinct larval populations whose varying behaviors differentially affect dispersal as density changes. On the other hand, Long's (1953) paper on two species of moth larvae indicates that behavior does not change as density increases, rather, the degree of behavior is altered. Similar results pertain to other papers discussed throughout this review.

In a series of papers, Surtees (1963a, b, & c; 1964a, b, c, e, & f) studied the dispersal of several species of grain weevils, grain beetles, and flour beetles in relation to environmental cues. The distribution, however, could not be fully accounted for until density effects were considered. A vertical distribution was discovered in the grain weevils Litophilus granarius, Oryzaephilus surinamensis, and Stophilus granarius (Surtees 1963a, 1964d). As the

density of weevils increased, the number of weevils aggregating at the surface of the medium increased. (These experiments are discussed in some detail under "Environmental Factors" and will be reexamined under "Biological Conditioning.") Surtees (1963c) also studied aggregations of Tribolium castaneum and Crystolestes ferrugineus, finding that mobility and aggregation behavior were affected by density, temperature, and moisture. There were, however, species differences. For example, surface aggregations of Tribolium were larger and more varied with density, whereas Crystolestes exhibited a more even vertical distribution at all densities. These aggregation responses Surtees interprets as modifications of individual locomotor activity which affects the intensity of intra-group stimulation and leads to upward dispersal and surface aggregations at high density.

In some instances it has been found that age of interacting larvae, as well as non-conspecific interactions, modify the effects of density. Salt and Hollick (1946), for example, found that not only was the spatial distribution of wireworm populations (Agriotes) related to physiological tolerances based on environmental cues, such as temperature and humidity, but to the presence of other organisms as well, such as ants. However, the developmental stage also affected conspecific interactions. Medium sized larvae showed a less marked aggregation behavior than did small (young) or large (old) larvae. No hypothesis or test of this phenomenon was offered.

Legay and Chasse (1964) hypothesized that dispersal is dependent on exploration and population pressure due to increasing density in Tribolium. They set up several "enclosures" in which they varied the

number of beetles and recorded dispersal behavior. Initially, beetles rapidly leave the enclosure, but the rate of movement declines as population pressure decreases. It appeared that tactile stimuli played an important role in this behavior, and lessened population pressure was interpreted in terms of lowered tactual stimuli. Interestingly the enclosure eventually always emptied. Legay and Chasse interpreted this to mean that exploratory behavior never reaches zero, even when population pressure is low. Although not thoroughly investigated, they observed a curious phenomenon. The mean duration of stay of individual beetles in the enclosure tends to increase with increasing density. According to Legay and Chasse, this is a result of accommodation to tactile stimuli among the beetles which had not discovered the opening of the enclosure. However, they failed to account for the fact that as density increases, the number of contacts increases which results in increased population pressure and movement. Theoretically, increased density should increase the probability of an individual finding the enclosure opening and dispersing, not increase the duration of stay in the enclosure.

A possible explanation for increased time spent in the enclosure as density gets high, is that the beetles are responding to biological conditioning. Legay and Chasse failed to take into account the literature preceding them which demonstrates conditioning responses in Tribolium. T. confusum prefers homotypic and heterotypic conditioning, whereas T. castaneum is repelled by both. A species comparison would have been very helpful in their studies. In the case of T. confusum, an increased mean duration of stay in the enclosure is to be expected

with increased density because the conditioning, which they prefer, is also increasing. The reverse should be true of T. castaneum. Such experiments would be necessary to separate out density effects from conditioning effects.

Zromiska-Rudjka (1966, from Ogden 1970) performed such a species comparison, but again conditioning effects were not accounted for. He looked at the effects of density on dispersal in T. confusum and T. castaneum in atrophic (roasted sawdust) and normal medium. Dispersal in the atrophic medium seemed to be independent of density, but if a small amount of normal medium is added dispersal becomes density dependent. He further found that dispersal is only slightly density dependent in T. confusum. This latter result may have been due to increased attraction to conditioning as density increases, whereas T. castaneum is repelled.

Some studies of dispersal indicate that there may be "trans-developmental-stage" effects of density. That is, the density of one developmental stage may affect the dispersion behavior of future stages. Long (1953) was interested in the connection between high larval (Lepidoptera) density and subsequent adult migratory behavior. His long range goal was to see if larval density was associated with color phases in the adults, as had been demonstrated in locusts. The results of his experiments show that crowded larval cultures develop more rapidly than solitary individuals, partly because the larvae go through fewer instars. If cultures became excessively crowded, however, development slowed. Long's basic theoretic was that behavior may be viewed as the outward expression of the ability of an organism

to respond to its internal and external environments. The prevailing pattern of behavior may therefore be considered as the exhibition of those responses being evoked at any one time. Thus crowding may lead to distinct behavioral changes. Crowding produces a change in behavior reflected in higher levels of activity which involves many non-feeding movements. Feeding activity in solitary larvae, however, is less.

When compared with the solitary condition, crowding represents a change of environment and accordingly may lead to a change in the frequency of behavior without necessarily involving a change in the basic behaviors. For example, the larvae of Plusia gamma, the British migrant silver Y-moth, in crowded cultures frequently show avoidance reactions to other larvae as well as warding off movements with the head and anterior region of the body. Isolated larvae occasionally exhibit non-feeding movements but are mostly inactive. If, However, a solitary larva is placed in a crowded situation, it exhibits behaviors typical of crowded larvae. Other differences in behavior occurred in solitary and crowded cultures, but the most striking was the general impression that, whereas the solitary larvae were relatively immobile, larvae in crowds were inclined to "restlessness." A higher activity level in high density situations is not surprising, as it is possible that the environment of crowded larvae initiates extra responses. Long also found that individuals in crowded cultures spent only one fourth as much time feeding as did solitaries. Thus, the extra activity is not just feeding but also entails locomotion, swaying motions, and irritation reactions between touching larvae.

In another study, Long (1955) attempted to demonstrate that the density of egg clusters is the main determining factor in larval dispersion behavior. However, his results are inconclusive because they were confounded by biological conditioning behaviors and a not very convincing species comparison. This study is discussed under "Biological Conditioning."

In the field, larval forms of certain Lepidoptera tend to be solitary and such a condition can be reproduced in the laboratory. In other species living in close aggregations, the population density considered normal for the species is not known. This leads to difficulties in interpreting the results of an experimental condition of isolation versus crowding as a departure from normal. This is also one of the main reasons I elected to approach dispersion studies with Galleria by beginning with isolates and gradually increasing group size. It also becomes necessary to be able to separate density effects from conditioning effects, which is best accomplished by first obtaining base line responses to conditioning before asking questions about animal-animal interactions. This is the major reason, in such studies as Long's, that it has not been possible to definitively elucidate the critical variables in spatial distribution.

Biological Conditioning, Defined

Every organism modifies its environment to some degree, whether it be the intricate structures built by spiders, birds, or bees, the making of burrows in aquatic and terrestrial forms, the secretion of simple structures such as slime tubes of marine annelids, trail marking in ants, scent marking in mammals, or hive odors in

bees. Such environmental modifications are classified as "biological conditioning."

Biological conditioning is ". . . changes produced in the medium by organisms living therein and the effects of such changes upon other organisms . . ." (Allee 1934). Allee's definition contains two parts. One relates to changes in the medium and the other to the effects such changes have upon other organisms. My studies are concerned with the effects of biological conditioning on conspecifics, not with the conditioning per se. Changes in the medium include chemical as well as physical changes. Allee et al. (1949) also refer to biological conditioning as "environmental conditioning" which they define as the modification of the environment by population-group activities. Biological conditioning can be homotypic or heterotypic. If the conditioning an organism responds to is produced by the organism itself or by a conspecific, it is homotypic. If produced by other than a conspecific, it is heterotypic. These definitions encompass any modification an organism makes in its environment. However, careful reading of the literature reveals an implicit attempt at parcelling or categorization of these phenomena into specific areas. This categorization seems to be based upon: (1) source of the conditioning, (2) medium in which the conditioning occurs, and (3) duration of the conditioning.

The source of the conditioning refers to whether it emanates from the organism or predominantly from the manipulation of environmental materials. The former is usually considered biological conditioning, in a strict sense, and the latter is often classified as

"external constructions" or "animal architecture," although neither category is exclusive of the other. Conditioning emanating from the organism refers to various organismic secretions and/or excretions. Falling into this category would be such things as spider webs (Kaston 1964; Witt and Reed 1965), quinone secretions by Tribolium (Alexander and Barton 1943; Loconti and Roth 1953), spinning behavior in Galleria mellonella (Paddock 1913; Milum 1952), chemotaxis in Paramecium caudatum (Dryl 1963), aggregation due to chemical stimuli in planaria (Reynierse 1967), and nest-entrance marking by honey bees (Butler et al. 1969). Examples of conditioning based solely on environmental materials are nest building in birds (Collias 1964) and various burrows dug by mammals. This dichotomy is not a discrete one, since conditioning might easily be a combination of environmental materials and secretions or excretions. Honey bee hives, termite nests, and caddisfly cases are combinations of the two.

The second consideration in biological conditioning relates to the medium in which the conditioning occurs. Most of the literature, classified as studies in biological conditioning, deals with conditioning in some sort of solid medium (i.e., soil, flour, or grain) or aquatic medium. This is true of most early work in the field, particularly Allee's studies, and of much of the recent work with such organisms as Tribolium, grain weevils, Galleria, and planaria. This also fits in with Naylor's (1959) concept of herbivorous organisms that find food and shelter in a fixed location. However, researchers do not include air in their concept of a biologically conditionable medium because the majority of conditioning

in air is chemical, which is usually considered chemical communication (see Johnson et al. 1970). At this juncture, it is necessary to point out that all chemical secretions are considered as biological conditioning, but not all biological conditioning is of a chemical nature, much of it is physical conditioning.

Many authors restrict biological conditioning to having long-lasting behavioral as well as physiological effects, and most air-borne conditionings are not of this nature, although no one has defined "long-lasting." Examples of these are sex attractants in phasmid moths (Taylor 1931), trail phermones in ants and other insects (Carthy 1951; Karlson and Butenandt 1959), and scent marking in mammals (Johnson 1973). This is not true, however, if the chemical conditioning is constantly reinforced. Besides being of relatively short duration, many air-borne phermones tend to have short term effects. These statements do not pertain to other than chemical signals, such as spider webs. On the other hand, conditioning of all types in a more solid medium tend to be more long-lasting than in air and have persistent behavioral and physiological effects. This is probably a function of the medium, not the stimulus.

A categorization of biological conditioning as to source, medium and duration is of little help in understanding the phenomenon, and has created problems for anyone interested in such studies. Part of the problem stems from the vastness of the area in which no real synthesis has yet been attempted. In fact, the last review of this area was written by Allee (1934). Another difficulty, as with any scientific area, is purely semantic. The major difficulty stems from

the fact that studies in biological conditioning originally were offshoots of other kinds of studies, and, from a behavioral point of view, have never come into their own as a field of investigation. Recently, however, there have been several excellent works dealing with reviewing some aspect of conditioning, such as a review of communication by chemical signals (Johnston et al. 1970) and a book on animal architecture (Von Frisch 1974).

It would be more useful to classify biological conditioning, in natural and experimental populations, on the basis of the processes involved. This seems a more practical point of view than attempting to categorize conditioning on the basis of the medium in which it occurs or how long-lasting it is. These are aspects of the problem, but purely artificial as a basis for classification. Allee et al. (1949) originally took such a practical approach and devised the following classification, with modifications. Biological conditioning consists of: (1) reduction and/or partial distribution of the available food supply; (2) addition of contaminants to the environment; (3) liberation of a "growth-promoting," or some other needed substance to the medium; (4) fixation by the population of toxic substances ("detoxification"); (5) osmotic regulation of an aquatic environment; (6) physical conditioning of the environment; (7) chemical conditioning of the environment; (8) compound conditioning, or combinations of categories 1-7.

Before proceeding to specific studies, the following generalities should be kept in mind:

1. Studies in biological conditioning entail studying the changes produced in a medium by an organism or group of organisms as well as the effects of such changes upon other organisms, particularly conspecifics.
2. These changes may be produced in any medium, i.e., soil, grain, flour, water, and air.
3. The changes produced can be physical or chemical and homotypic or heterotypic.
4. Chemical conditioning in air and physical conditioning in any medium are usually classified as "Chemical Communication" and "Animal Architecture or Construction," respectively. However, both of these phenomena come under the broader classification of biological conditioning.
5. The effects of changes in the medium upon other organisms may be physiological, morphological or behavioral and of varying duration.
6. Biological conditioning does not solely entail adding something to the medium. It may also be a rearrangement of the medium or removal of substances from the medium (detoxification).
7. Biological conditioning can be produced by an individual or by groups of individuals, and therefore is not a purely population phenomenon. In this context, animal-animal interactions become very important.

The remainder of this review will deal with some of the classic work in biological conditioning, exemplifying the various categories of conditioning as well as illustrating its physiological and behavioral

effects. The physiological and behavioral effects of conditioning will be discussed in the context of animal-animal interactions. This is particularly relevant in light of my studies in this dissertation which are concerned with the function of biological conditioning in relation to spatial distribution, and how preferences for conditioned medium are affected by animal-animal interactions coupled with development (age) of Galleria mellonella larvae.

Physiological Aspects of Biological Conditioning

Most of the early literature deals with biological conditioning in relation to the physiological changes produced in various organisms. These studies were classified as studies in "Mass Physiology" which Allee (1934) defined as the result of the aggregations of many individuals of the same species in a limited space. An excellent review of this concept is also found in Allee's (1931) book, Animal Aggregations. Basically, studies in mass physiology deal with density and biological conditioning as they affect reproduction, fertility, fecundity, natality, mortality, and growth. For example, Edmondson (1945) found that growth in several species of sessile rotifers was affected by crowding. Isolates were larger than colonial forms, possibly a result of competition for food, but aggregates began reproducing sooner and lived longer than solitaries. Edmondson's conclusion was that crowding acts to enhance survival.

Studies in mass physiology deal with (1) protection from toxic substances, (2) control of sex, (3) effects on morphological changes, (4) effects on growth, and (5) effects on population dynamics.

Protection From Toxic Substances

Often a group of organisms will yield each other protection from a toxic environment to which animals exposed singly would succumb. The turbellarian flatworm (Procerodes wheatlandi), for example, survives longer in fresh water (it is normally an intertidal dweller) if present in large numbers and if the water has been previously homotypically conditioned. This conditioning is particularly effective if it consists of some dead and disintegrating flatworms (Allee 1931).

The greater the number of Planaria dorotocephala in a group, the greater the survival of individuals after exposure to colloidal silver (Allee and Schuett 1927). Similar results have been obtained with P. maculata and P. lacteum as well as with the brittle starfish Ophioderma brevispina. The greater protection which is afforded by the mass when exposed to colloidal silver is probably due to the smaller amount of the toxic substance which each individual removes from the water in order to lower the strength below the threshold of immediate toxicity. No evidence has been found for the presence of an "auto-protective" or "auto-destructive" agent which would protect individuals from toxic substances. Evidence did indicate, however, that slime secretion may remove the silver and render the solution less toxic. Aggregations of mixed species also exhibit the protection from colloidal silver.

Perhaps the most classic study of mass protection from toxic conditions is Allee and Schuetts's (1927) study on the relation between mass of animals and resistance to colloidal silver. Tests were performed on a variety of organisms such as planaria, brittle

starfish, isopods, and goldfish. In all cases the results show a greater protection from toxic substances the greater the mass of animals present.

It is not necessary to further demonstrate that mass protection is a common phenomenon. However, the mechanism has also been investigated. According to Allee (1934), four theories have been proposed to account for mass protection:

1. Groups of organisms more effectively produce auto-protective substances which in some manner conditions the medium and protects the group (Drzewina and Bohn 1921).
2. The mass of animals more rapidly absorbs or exhausts the toxic agent, so each animal receives a smaller dose (Allee and Schuett 1927; Breukelman 1932).
3. Grouped animals reduce their metabolism, as measured by oxygen consumption, compared with isolates. This reduction favors survival in toxic solutions (Allee and Fowler 1932).
4. The group may be protected by altering the electrical conditions (Drzewina and Bohn 1926), or animals may be mutually influenced by the presence of other animals without the diffusion of any substances.

All of the evidence collected by Allee favors the assumption that the mechanism, at least as it relates to colloidal silver, is protection due to the more rapid removal of the toxic element by the mass of individuals or their products. The evidence further indicates that no other factor need be assumed to account for protection from other substances. However, in most of Allee's studies the

organisms also exhibited a general reduction in metabolism. No one has attempted to decipher if the conditioning merely brings individuals into a group and the group then, not the conditioning, provides protection from toxic substances.

Control of Sex

Attempts have been made to determine the relative role played by food, crowding, and conditioning in relation to control of sex, but the most classic attempt was a series of papers in the early 1900s relating to control of sex in Cladocera (daphnids). Cladocera is normally parthenogenetic, but under certain conditions males are produced. According to Allee (1934) it had been hypothesized that crowding of the females in Moina rectirostris produces a higher percentage of males, and experiments testing percent of male offspring from female isolates versus female groups seemed to confirm this hypothesis. Initial conclusions were that the results were due to an increase in excretory products in the food, but further tests did not verify this. For some time hypotheses fluctuated between a food explanation and an explanation based on increased male production due to female excretory products. Earlier work supported the food hypothesis. Issakowitsch (1905, from Allee 1934) found he could change the form of reproduction in Cladocera from parthenogenetic to bisexual by manipulating food supplies, and similar studies indicated that repeated transfers to fresh culture water rich in food delayed the appearance of bisexual forms. Metabolic wastes did not appear to affect this transformation and thus the effect was attributed to decreasing food supplies. Smith's (1915) work, however, supported the excretory product

hypothesis. He grew cultures of Protococcus on excess food in crowded conditions and found excess of male production. He concluded that crowding exerts its effect through some sort of excretory product. Stuart and Banta (1931) demonstrated a relationship between the bacteria present in Cladocera cultures and male production. Male production increases, within limits, as the number of bacteria decreases, and there were no indications that the bacteria were absorbing excretory wastes.

It seems obvious, in retrospect, that sex control is probably a multiple effect of food, crowding, and conditioning, and that the mechanism may be different from species to species. Brown and Banta (1935) postulated such an interaction and their results seem to support it. They found that the amount of food acts as a broad or limiting factor in male production, whereas crowding of females and excretory products act as immediate factors to increase male numbers. This series of experiments exemplifies reduction and/or partial distribution of available food supply and addition of contaminants or some needed substance to the medium as types of biological conditioning.

A second example of how crowding, and possibly conditioning affects sexual differentiation comes from Loomis and Lenhoff's (1956) work with hydra. Clonal growth within a mass culture of hydra follows a segmoid curve and sexual differentiation occurs spontaneously in growing clones whenever a critical level of population density is reached. This "positive group effect" results from the differentiation-inducing activity of a volatile chemical or gas secreted by the hydra

themselves, and the secretion may be a reaction to animal-animal interactions at increasing density.

Effects on Morphological Changes

One of the best known examples of the effects of mass physiology on morphological changes relates to crowding and the phase theory in locusts. Uvarov (1928) proposed the theory that species formerly supposed to be monomorphic are in fact polymorphic as regards coloration of the nymphs, form of the pronotum, body size, and relative wing length of adults. A "gregarious phase," which demonstrate a tendency to migratory behavior, and a "solitary phase," which lacked such behavior, were identified in locusts. The gregarious phase is darkly colored and the solitary phase is green. Uvarov suggested that locusts pass from one phase to the other, depending on population density. This theory has since been well worked out in locusts and other species of Lepidoptera (Long 1953, 1955; Doull 1953; Ellis 1953). However, it was Faure (1932) who first demonstrated the mechanism. Crowding causes increased activity and the resulting increase in metabolism deposits end products in the cuticula giving the gregarious phase its dark color.

Effects on Growth

It has been repeatedly demonstrated that density affects growth rate. Allee (1931) reviewed the early literature on the effects of space limitations on growth rate and Adolph (1931) noted that crowding in Rana sylvatica tadpoles causes an earlier decline in growth rate than in isolates. If isolated after having been crowded, growth

rate increases. Similar studies in "growth depensation" were run by Brown (1946, 1951) with trout fry, Magnusen (1962) with Medaka, Peebles (1929) with echinoderm larvae, and Richards (1958) and Rose (1959) with tadpoles, all of whom found growth effects produced by grouping of these organisms. Hypotheses were advanced that growth differences were due to either spatial, numbers, or conditioning relationships, but none of these was clearly indicated. These are probably cases of multiple interactions, but the usual approach has been to single out a variable of interest and examine its effects on growth.

Homotypic and heterotypic conditioning of the environment and their effect on growth was studied by Livengood (1937) and Allee et al. (1934, 1940) in goldfish, Shaw (1932) in fish and amphibians and West (1961) in tadpoles. Most such studies concluded that growth is most rapid in "mildly" conditioned medium and retarded in overcrowded and/or over-conditioned medium. Excretions or depleted food supplies were postulated as the mechanisms, but never demonstrated. Adolph (1931) decided that growth retardation in crowded conditions was due to "psychological" effects, not physiological effects. That is, individuals in crowded cultures eat less but live longer than do isolates. On the other hand, Welty (1934) found just the opposite in fish. Crowded fish ate more than isolates but grew less. However, Welty did not replenish the culture water in his experiments and his results may have been due to growth-retarding contaminants or to group stimulation to greater activity. In 1921, Robertson gave the name "Allelocatalysis" to the process of added "contaminants" affecting growth.

Effects on Population Dynamics

Aside from growth effects, mass physiology also affects fertility, fecundity, mortality, and natality. Perhaps the best worked out example of mass physiological effects on population dynamics is that begun by Pearl and Chapman and pursued by Park with Tribolium. These studies also serve as the basis for most of the work on biological conditioning and behavior.

Pearl (1927) showed that various populations follow a logistic growth curve and postulated that increasing density affects growth by affecting natality and mortality. Chapman (1928) tested this hypothesis with Tribolium confusum and found that an equilibrium condition, measured in terms of numbers of individuals per gram of flour, is eventually reached, within each population, which is uniform regardless of environmental size or initial densities.

Thomas Park (1932) first began his experiments with Tribolium confusum by asking whether the initial rate of population growth is greater in beetle populations when more than one pair of beetles is present. He utilized constant sized environments but variable numbers of pairs per vial (i.e., 2, 4, 8, and 16 pairs). Measurements of population growth were taken at 11 and 25 days. At 11 days, the most eggs had been produced by the initial population of 2 pairs and the fewest by 16 pairs. At 25 days, this relationship had reversed. Eventually the populations reach the same equilibrium, regardless of the initial starting density. Further studies enabled Park (1932) to elucidate the mechanisms producing these findings. Intermediate sized Tribolium populations grow more rapidly at 11 days than smaller or

larger populations because (1) egg cannibalism favors greatest increase in minimal sized groups since in crowded groups more eggs are found and eaten by random movement, and (2) copulations and recopulations favor greatest increase in maximal sized groups, recopulation being stimulating to reproductive productivity. The interaction between egg cannibalism and copulations thus favors the greatest initial increase in intermediate sized populations.

As populations of Tribolium inhabit their flour, the flour becomes progressively more conditioned with time. The extent of this conditioning is proportional to the population density. Noticing this phenomenon, Park (1934, 1935, 1936 a and b), Park and Woollcott (1937), and Park et al. (1939) began a series of investigations into the effects of "auto-conditioned" flour on populations of Tribolium. The reader is directed to Park and Woollcott (1937) and Allee et al. (1949) for excellent summaries of these studies, only the specifics will be presented here.

Conditioned flour was found to affect beetle physiology. Conditioning reduced egg cannibalism and fecundity, increased egg viability and duration of larval development, and seemed to have no effect on egg fertility or the rate of oxygen consumption of adults. Dilution experiments, in which heavily conditioned flour was graded by adding variable amounts of fresh flour, showed that beetle fecundity is proportional to the amount of conditioned medium. Conditioning also affects metamorphosis. Larval crowding increases larval and pupal mortality and conditioning seems important here. However, the duration of the pupal period is not altered by density, sex, or conditioning.

There are two general conclusions possible from Park's studies with Tribolium: (1) flour becomes progressively conditioned as populations age, and (2) Tribolium populations decline as they age if their flour is not renewed. Therefore, conditioning is an expression of population age and size and the number of beetles is responsible for the rate and amount of conditioning. It seems fairly clear that the decline of Tribolium cultures is largely due to reduction in reproductive rate as a function of conditioning. Even today, however, it is not clear what constitutes conditioning but it seems reasonable to assume that it is a combination of food depletion and addition of toxic waste products. Park and Burrows (1942), for example, have shown that fecundity is sensitive to changes in the nutritive level of the medium, and Roth and Howland (1941), Roth (1943), and Loconti and Roth (1953) have demonstrated that crowded imagoes liberate a gas from specialized odoriferous glands which causes morphological abnormalities. Crombe's (1942) study lends further credence to Park's data by demonstrating that fecundity is altered by conditioning.

In light of Christian's (1970) hypothesis dealing with changing behaviors as density increases, the results of Park's work might be interpreted as changes in the degree of egg cannibalism and reproductive behaviors as population density and conditioning increase.

Biological Conditioning and Spatial Distribution

Other than having physiological and demographic correlates, biological conditioning is associated with numerous behavioral responses in individuals and populations. Chemical communication, as

reviewed by Johnston et al. (1970), is an outstanding example of the behavioral relationships of conditioning. Chemical conditionings (specifically phermones) are often classified as releasers, chemical stimuli initiating immediate behavioral responses, and primers, chemical stimuli inducing delayed responses (Wilson and Bossert 1963). Chemical stimuli have been found associated with recruitment in ants (Karlson and Butenandt 1959; Regnier and Law 1968), sex attractants (Silverstein and Rodin 1967; Jacobsin et al. 1970; Silverstein et al. 1967; Regnier and Law 1968), and alarm reactions (Maschwitz 1964; Pfeiffer 1963).

Biological conditioning by means of chemicals is a very common phenomenon among the animal phyla. It has been well studied in mammals under the heading of "scent marking." Although scent marks may have any number of functions, one hypothesis is that they serve to warn conspecifics of an already occupied territory and function to keep the population spatially dispersed. Our main concern in this review, however, is with the invertebrates, particularly insects, and the reader is directed to Johnson (1973) for a review of chemical conditioning in mammals.

Various physiological and demographic effects of conditioning have been illustrated, but the relationship between biological conditioning, animal-animal interactions, and dispersion is not as well worked out. Biological conditioning has historically been thought of as a population phenomenon since it is an expression of population density and is a normal and inevitable result of population growth. As populations grow and maintain themselves they must of necessity

modify their environment, and the environment in turn modifies them. Since biological conditioning is a result of the organisms themselves and since the degree of conditioning is a function of the number of organisms present, biological conditioning and animal-animal interactions will be considered as a unit.

One of the classic ecological studies is Edmundson's (1945) paper on sessile Rotatoria. He found that the distribution of several rotifer species is partly dependent on responses to heterotypic conditioning by the plants upon which they settle. Some rotifers, i.e., Floscularia conifera, seem to respond to homotypic conditioning as well. That is, they are found more abundantly attached to each other than to the plants. However, this may have been a tactual response and not a conditioning response at all. Heterotypic conditioning by algae and texture of the settling surface were found to be important to the spatial distribution of oyster larvae (Gee 1965). Cole and Knight-Jones (1949) had earlier discovered that oyster larvae tend to be gregarious on settlement and prefer to attach themselves when they have found a surface of suitable texture, a surface that had been homotypically conditioned by other larvae, or a surface where other larvae were already attached. Knight-Jones and Stevenson (1951) found basically the same responses exhibited by the barnacle larvae Elminius modestus. The conditioning response and response to properly textured surfaces may be linked. That is, conditioning may consist of physically modifying the settling surface.

When a species is found to exhibit some sort of predictable distribution, researchers begin a search for the mechanism(s) involved.

The work with planaria is a good example of the procedure. Planaria form large aggregations (Allee 1931). Fraenkel and Gunn (1961) suggested that aggregations may form mechanically by means of an orthokinesis, where rate of movement depends upon stimulus intensity, or a klino-kinesis, where rate of random turning depends upon stimulus intensity. This explanation of aggregation behavior was made in reference to phototactic responses. However, Reynierse (1967) found that planaria secrete some substance, possibly species specific, to which conspecifics are positively attracted. This form of biological conditioning was interpreted as inconsistent with Fraenkel and Gunn's requirement that aggregations are accidental and mechanical results of a kinesis. However, chemical secretions may affect both the rate of movement and rate of turning. A similar study was performed by Dryl (1963) with Paramecium caudatum in which he found them maintaining their spatial distribution by an avoidance reaction to chemotactic stimuli. But none of his data indicate whether the Paramecium were secreting the chemical.

There are currently three hypotheses in vogue relating to aggregation formation in invertebrates, specifically in planaria:

1. Aggregation formation is due to the secretion of some chemical substance to which conspecifics are positively attracted (Walter 1907; Allee 1931; Reynierse 1967).
2. Aggregations form mechanically as a result of an orthokinesis or klinokinesis (Frankel and Gunn 1961).
3. Aggregations form as a result of distinctive morphology. That is, an aggregation forms a distinctive visual or tactual

stimulus to which conspecifics respond (Reynierse et al. 1969).

Using various degrees of illumination and natural as well as artificial planaria aggregations, Reynierse et al. (1969) found support for all three hypotheses. In other words, aggregation behavior will only rarely be explainable on the basis of single mechanism hypotheses. This appears to be a truism, but it is an extremely important consideration for studies in spatial distribution and accounts for much of the conflicting and inconclusive results seen in the literature.

The most well worked out studies dealing with biological conditioning and animal-animal interactions are those stemming from Park's work on Tribolium. We have already seen the physiological effects of conditioning in Tribolium confusum as worked out by Park (1934, 1935, 1936a and b), Park and Woollcott (1937), and Park et al. (1939). In his 1934 paper, Park provided adults with a choice between fresh (unconditioned) flour and two year old (conditioned) flour, and noted that, besides the effect on egg production and fecundity, adults avoided the conditioned medium. Therefore, the physiological consequences of conditioned medium are probably not a result of adult preferences for over-conditioned medium. Park (1948) further investigated competition between Tribolium confusum and Tribolium castaneum, finding that both species cannot live together for extended periods of time, and if forced to do so, Tribolium castaneum is the successful competitor. The results of such competition may be due to differential conditioning of the medium, causing the reduction of available food, the elaboration of environmental poisons (i.e., excrement and

carbon dioxide), or comminution of the flour itself. These possibilities were not tested.

Park assumed the movement of flour beetles to be random. In 1932, Stanley analyzed the movements of Tribolium confusum on the basis of the kinetic theory of gases, considering the beetles as particles having random movement. He found that the predictions of randomness were not consistent with experimentally observed populations, and hypothesized that this assumption is not valid because the beetles are leaving persistent tunnels in the flour and the probability of re-traversal of a tunnel is greater than that of making a new tunnel. Using a theoretical organism, Stanley (1949) mathematically demonstrated that the probability of re-traversal of existing tunnels increases with time whereas the probability of constructing new tunnels decreases. In theory, as time approaches infinity, the whole flour mass becomes converted into old tunnels, all traversed an infinite number of times. In actual practice, tunnel making stabilizes at some point before this occurs because the beetles prefer using existing tunnels. Observations on Tribolium seem to confirm Stanley's prediction that spatial distribution is a function of biologically conditioning of the medium (tunnels). However, his theory does not account for animal-animal interactions which may be a part of the tunneling behavior observed.

Some of Stanley's observations also shed some light on the behaviors involved in the egg cannibalism observed by Park. It was discovered that eggs are laid in the tunnels and as population

density increases, egg cannibalism increases as a result of the adults retraversing existing tunnels.

Rich (1956) confirmed these observations and states that population ecology is, in the final analysis, concerned with the interaction of natality, mortality, and dispersion. These three factors produce shifts of numbers over time in a reproducing population. In Tribolium, however, these shifts in numbers are not constant over time and egg cannibalism is probably a function of number of adults present, egg distribution, and the pattern of beetle movement.

One of Park's students, Naylor (1959), made the next major contribution to understanding the relationship between biological conditioning and animal-animal interaction in the spatial distribution of Tribolium. He was interested in the behavioral mechanisms of dispersal, as distinct from natality and mortality, in Tribolium confusum populations, and was one of the first to devise a choice situation. His apparatus consisted of a circular device with vials of flour around the periphery. The number of vials could be varied as well as the flour content of each vial. Beetles were placed in the center of the apparatus and allowed to choose the preferred environment. Statistical analysis enabled him to distinguish between uniform, variable, and random distribution after choices had been made. Several experiments were performed using varying densities of populations and the results show that at low densities the beetles either aggregate or respond to minor differences in the environment. As density increases, "population pressure" overcomes the influences producing aggregation and forces uniform patterns of distribution.

Naylor's (1959) next series of experiments were designed to determine what behavioral factors were involved in establishing these distributions. He first examined male-female interactions, and found that females spend more time in the flour than do males. At all densities and in mixed or uniform sex populations, females exhibit a uniform distribution whereas males aggregate. Both sexes also preferred conditioned medium. The males' preference for conditioned medium is consistent with their aggregation tendency, but the same preference on the part of females is inconsistent with their uniform distribution. The conditioned vials, however, were initially free of other individuals and it is possible that the females would not have aggregated in conditioned medium had other beetles been present. To test this hypothesis, it is first necessary to demonstrate that the beetles are capable of behavioral responses to other individuals. Naylor ran several preference tests to determine this. If given a choice between vials with trapped beetles of either sex and vials of only fresh flour, males in single sex groups enter the chambers with trapped beetles. If the choice is between male or female chambers, males prefer females. Females, on the other hand, selected non-occupied chambers, but if all vials contained trapped beetles they showed a greater tolerance for males. These experiments demonstrate that beetles behaviorally respond to other individuals but do not explain why females aggregate in conditioned medium, unless conditioning overrides their tendency to be repulsed by other females. This was not tested.

In 1965, Naylor ran a similar set of experiments except larvae were used as trapped animals. Females avoided these vials and the avoidance was density dependent. The same experiments were also run with Tribolium castaneum (Naylor 1961). The results were similar except that spatial distribution was not as markedly affected by density and male T. castaneum is repulsed by conditioning.

In general, if sex is ignored, the spatial distribution of Tribolium is dependent on density. But if sex and conditioning are introduced as variables spatial patterns are altered. Naylor (1959) attempted to fit these findings into some kind of population model. Females enter the vials to feed and oviposit. By distributing themselves uniformly, few suitable sites are overlooked and few would be crowded by hatching larvae. This would increase the probability of larval survival. It would also be advantageous for females to be able to respond to levels of occupancy of the habitat and make appropriate adjustments in dispersal. If females were capable of such behavioral responses, they might form low density aggregates but disperse if density became too great. One factor Naylor's model does not account for is that inbreeding may be promoted at low densities which may not be advantageous to the population.

Naylor's (1961) paper was the springboard for a series of articles dealing with species comparisons of animal-animal interactions and biological conditioning. In 1963, Ghent contrasted the behavioral responses of Tribolium confusum and T. castaneum to fresh and conditioned flour and attempted to assess the influence of these responses upon distribution, population growth, and competition between the two

species. A vertical distribution exists in these species dependent upon sex, density, and conditioning. T. castaneum prefers fresh flour and T. confusum prefers conditioned flour, regardless of sex or type of conditioning. T. confusum is attracted to homotypic and heterotypic conditioning, whereas T. castaneum is repelled by both, and these preferences are influenced by population density. As density increases, the proportion of the total population that the preferred niches will tolerate decreases. Therefore, at high densities the distinction between preferred and non-preferred niche becomes hazy. With increasing density, females of both species show a greater tendency than males to enter a non-preferred niche. The increased density seems to "drive" beetles to the surface of the flour where the males normally remain. Females, on the other hand, chose to enter a non-preferred flour medium. Female T. castaneum will enter conditioned medium rather than stay on the surface, and female T. confusum enters fresh flour. It is also generally the females that come to the surface at high densities, not the males. This indicates that the males are forcing them upwards, as Naylor (1959) suggested, or that the females are somehow sensing their environment and moving to less dense areas. Ghent postulated that these behaviors represent an adaptation that allows aggregations of T. confusum to follow in the occupancy of a niche as a successor to some species like T. castaneum that becomes progressively more repelled by the accumulating conditioning. Since Park (1948) demonstrated that T. castaneum out-competes T. confusum, this may be a mechanism to avoid competition.

In 1966, Ghent pursued this hypothesis by bringing the two species together and asking whether the resultant distributions would be as predicted from the preceding facts. He placed a population of each species in a fresh flour medium and found the following: Both male and female T. castaneum entered the flour first and began a downward migration. Male and female T. confusum entered more slowly, possibly as a result of fresh flour being their non-preferred medium. However, female T. confusum enter more readily than do the males, as found in Ghent (1963). A vertical stratification is thus established, with male T. confusum near the surface, female T. confusum in the middle, and male and female T. castaneum on the bottom. As the male and female T. castaneum conditioned the bottom flour layer, they became repelled by it and started a migration back up to the surface. At the same time the male and female T. confusum are attracted by the heterotypic conditioning and migrate downward, the conditioning having overcome male T. confusum's inhibitions about entering the flour. The two species populations cross somewhere in the middle of the flour. Concomitant population dynamic data showed that initially egg production and survival were higher in T. castaneum until repulsion to conditioning set in.

On the basis of this experiment, Ghent's (1963) model, that the preferences of T. confusum represent a mechanism for escaping competition, permitting succession in occupancy of previously unexploited environments, seems tenable. One may envision a species like T. castaneum being increasingly repelled by a niche as it becomes conditioned and abandoning that feeding niche before its nutritive value

is fully exploited. The availability of such an incompletely exploited niche may then have furnished the element of survival value that promoted the selection of conditioned flour preference among ancestral T. confusum. Ghent beautifully supports this theory with an argument drawn from the plant kingdom. The larch (tamarach) grows best on relatively dry, rich uplands. Yet, as a continuous population, larch is found commonly only in swamps where its growth rate is substantially less. The upland sites are normally taken over by other species with which larch cannot successfully compete and which, in turn, grow poorly or not at all in swamps. Given a choice between extinction through competition or survival, larch adapted its requirements to live in swamps.

Surtees (1963a, b, and c; 1964a, b, c, d, e, and f) investigated the spatial distribution of several species of grain weevils and Tribolium (see the "Environmental Factors" and "Density" sections of this review). The basic finding in all these studies was that when groups of insects are confined at high densities a certain species-specific percentage of organisms was found at the surface of the medium. Coupling these density effects with what he had found in relation to temperature and humidity, Surtees (1964c) developed the following theory of spatial distribution. Mutual disturbance due to individual movement elicits negative geotaxis and this plays an important role in determining group dispersion. Although such disturbances cause an upward movement in restricted experimental populations, this would result in the spread of a population in a larger grain bulk firstly by upward movement and then by more random movements

resulting in colonization of hitherto uninfested grain. Aggregation, on the other hand, is a result of environmental stimuli modifying the generalized pattern of movement, including the irregularity of pathway or intensity of turning (klinokinesis) and the speed of movement (orthokinesis) which in turn reduces the disturbance factor and therefore dispersal pressure. Thus, aggregation may be expected in regions of lowest velocity. This latter conclusion was also made by Gunn and Pielow (1940).

However, Surtees seems to have ignored the literature before him, particularly Naylor's (1959) and Ghent's (1963) papers on Tribolium which show that many of the responses Surtees describes are due to conditioning effects. Of course, Ghent's (1966) paper tends to negate many of Surtees' conclusions, which must be very tentatively interpreted.

While research was progressing on the relationship between biological conditioning and dispersion, other researchers were attempting to elucidate the components of conditioning. Park (1934, 1935, 1936) had described the physiological and ecological effects of conditioning but had no idea what the effects were due to. Loconti and Roth (1953) and Ghent (1963) showed that a major stimulus to dispersal and aggregation in flour beetles is olfactory, related in some way to the conditioning of the medium. Some possible components of this conditioning are quinones, feces, casts, nutritional depletion of the medium, presence of fungi or bacteria and presence of larvae and eggs, although the principal component was suspected to be quinones (Ghent 1963).

Ogden (1969) set out to determine the important components of conditioned medium and to assess their relative importance in the dispersion responses of Tribolium. He gave adults choices between fresh flour and flour having some suspected component of conditioning. He utilized T. confusum and the conditioning was done by adults and larvae (larvae do not produce quinones). The results are the following:

1. T. confusum adults are attracted to medium conditioned with a series of adult densities as well as to medium conditioned by msg mutants (msg mutants produce very little quinone).
2. Adults are attracted to larval conditioning and to medium containing frass (larval excreta).
3. Adults are not attracted to medium containing eggs.
4. Adults are repelled by beetle quinones and give a variable response to commercially prepared methyl paraquinone, a component of beetle secretions.

It would appear that quinones are not the important component of conditioning, which is in direct contradiction to earlier studies. However, this debate is as yet unsettled. It is possible that quinones in low concentrations attract adults and quinones in high concentrations repell them, causing dispersal when population density becomes great. Ogden's (1970) paper however, does not seem to support this hypothesis.

Ogden (1970) compared T. confusum and T. castaneum with respect to dispersal, using Prus' (1963) apparatus described earlier. His results are consistent with early findings. T. castaneum disperses rapidly from homotypically and heterotypically conditioned medium,

whereas T. confusum aggregates in both. However, Ogden also found quinones not to be important to these responses. It was found that medium conditioned by a "little-quinone-producing-mutant" elicited the same responses from T. castaneum and T. confusum.

My own studies with Galleria conditioning make no attempt to assess the components of conditioning eliciting dispersion behavior, although a tactual response to silk is probably involved. This does not, however, negate the possibility of chemotactic responses.

The effects of biological conditioning and animal-animal interactions on spatial distribution might more clearly be seen in larval populations free from adult-young and sexual interactions. Lepidoptera populations, i.e., Galleria, provide us with such models but, for the most part, have not yet been well worked out. One of the earliest observations pertaining to these variables in a Lepidoptera population was made by Brindley (1910) on the behavior of the processionary caterpillar Cnethocampa pinwora. The larvae seemed to "march" in single file when on the move and formed a "circulating mass" just before burrowing into the soil to pupate. This circulating mass frequently formed and then dispersed. After massing, a new procession developed with the old leader usually still leading. Brindley concluded that in any procession one larva has a "greater tendency to stray" than the others and this one is more likely to act as a leader. (Wellington, 1957, extends this idea to tent caterpillars as will be seen shortly.) These larvae are constantly laying down a silk thread as they march in single file. Since Brindley could not elucidate a function for this behavior and since these threads

contained nitrogenous products, he attributed the behavior to excretion. Edwards (1910), however, noted that the single file processions consisted of larvae arranged in head-to-tail contact. The whole mass moves along a silken thread which is commenced by the leader and added to by all the larvae in succession. The number in the procession is variable, but can be up to 100 larvae in length. Any larva could be a leader, but once a particular larva begins leading it continues to do so. At night the procession returns to the nest. Upon approaching the nest, the leader searches until encountering one of the many silk strands emanating from the nest and follows it with the procession behind him. The leader exhibits a choice in this selection of a silk nest-strand in that it would not select artificial strands. Edwards further found that procession integration was helped by the silk strand but the predominant integrating force was head to tail contact. The silk strands were more important in relocating the nest after a feeding excursion, the silk strands being predominantly used in finding the nest.

The spinning of silk strands or tunnels is a common phenomenon in Lepidopterans, as well as other invertebrate orders such as Diptera, i.e., black-fly larvae, and Trichoptera, caddisflies. Silk trails may have much the same significance as scent-trails in mammals. Accumulation of webs and silk trails over the foliage and ground or to and from refuge nests may indicate how heavily an area has been travelled, which in turn could be weighed against the available resources. Perhaps the result of such behavior conditions the habitat in the same manner as chemicals do. Wynne-Edwards (1962) says that behaviors are

a form of epideictic display. For example, he postulates that the "circulating mass" behavior of the processionary caterpillars is a mechanism supplying information on population density. This has yet to be tested.

There is some evidence that the larvae of Galleria mellonella (the greater wax moth) condition their medium by means of silk trails and frass (Milum 1952). Newly hatched larvae at first feed on isolated portions of honeycomb in a bee hive, or beneath the comb's surface where they form silk tunnels covered with frass and bits of comb. Gradually these larvae assemble in a mass of webbing, with tunnels extending throughout the remainder of the combs in search of food. Oertel (1962) conducted tests to find if Galleria adults are attracted to old (larval conditioned) hives. He discovered that wax moths find the conditioned combs in preference to non-conditioned ones and concluded that odor from the old combs was attractive to them.

The social behavior of certain insects, such as ants, bees, and termites, has attracted considerable attention. However, such a "high-order" of behavior is restricted to very few species, the others existing in states from pure solitariness to sub-social aggregations. Lepidopterous larvae afford many examples covering the extremes of this range. Those leading a solitary existence generally do so as the result of the habit of the adult in laying eggs singly or in pairs rather than as a distinct anti-social facet in larval behavior. In other cases where eggs are loosely scattered or tightly clustered the opportunity for aggregation will exist (Long 1955). In a series of studies, Long (1955) set out to compare the "sub-social" behavior

concerned with aggregations in two species of caterpillars, Plusia gamma and Pieris brassicae.

Pieris brassicae (the large white butterfly) lays its eggs in tightly packed groups. The first larvae to emerge help the others to hatch by eating the tops off of the eggs. Once a large hatch has occurred, the larvae begin spinning silk which is of prime importance in their aggregation formation. Eventually a particularly active larva wanders off to an adjacent leaf, spinning a silk trail as it goes. The other larvae follow the trail. As they eat and spin the whole area is covered with a mat of webbing, and once this mat is formed future spinning is restricted to the mat or to fresh leaf areas. Accompanying spinning behavior is a distinct activity rhythm. The larvae feed and rest on common "mat sites." As they feed they chew a circular hole in the leaf, extending the mat as the hole gets bigger. After feeding they return to the mat and then push out again to feed. This mass behavior seems to depend on a few initiating larvae and their ability to transmit a sense of activity to other larvae. By about the fourth instar, the large aggregation begins breaking up into smaller ones, as a function of the food source, and individual feeding rhythms are altered.

Larvae raised in isolation were able to join established aggregations. In the first two instars solitary larvae could join an aggregation by following a silk strand to the group, and were allowed to join the aggregation only if their size was equivalent to the individuals in the aggregation. By the third instar, however, solitary larvae exhibited investigatory movements, whereas aggregations

remain passive. These investigatory movements interfered with the normal pattern of group behavior and joining a group became difficult. It became impossible for fourth instar isolates to join established aggregations because their individual activity rhythm was asynchronous with the group's.

Plusia gamma (the silver Y-moth) on the other hand, lays its eggs singly or dotted on the undersides of leaves. Regardless of this behavior, the eggs laid by a single female hatch in the span of a day and the opportunity for aggregation formation is present. Unlike Pieris brassicae, however, the larvae of Plusia gamma exhibit random movements, no noticeable silk spinning behavior, and a definite non-gregarious tendency. If given the proper circumstances, however, Plusia gamma larvae behave similarly to Pieris brassicae larvae. If given crowded experience they aggregate and eat more than isolates. A larva that was reared in a crowd and then allowed to roam free in a normal situation has a tendency to stop moving and begin feeding upon encountering another larva. Isolate reared larvae do not show this tendency, move more, and eat less.

Long concluded, therefore, that the large aggregations in Pieris brassicae depend on silk spinning for its formation and partly for its maintenance, whereas Plusia gamma has no such mechanism and forms aggregations only upon chance meetings. In both species, however, the ultimate factor in aggregation behavior was larval contact. Larval contact could stop a wandering larva or start larvae moving, although in Pieris brassicae the movements are both silk and larval contact directed. There is also an age variable in relation to

aggregation formation, particularly in Pieris brassicae. Larvae not of the same size (age) as those in the aggregation had difficulty joining the aggregation predominantly because of asynchronous activity rhythms.

Long's hypothesis about aggregations being a function of the size of egg clusters is unfounded. There is a correlation between eggs laid in clusters and those laid singly in the two species examined, but a sample size of two is not statistically sound. The larvae of Plusia gamma may not have formed aggregations because they hatched from eggs laid spaced out, but rather because they exhibit no spinning behavior which was the main cause of aggregation formation in Pieris brassicae. To further test the relationship between egg cluster size and aggregation behavior we would need a species that hatches from eggs laid singly and whose larvae spin silk trails.

The relationship between biological conditioning and mobility (activity) of organisms, as seen in Long's (1955) paper, is a frequently occurring one in the literature. In Lepidoptera, this relationship has been most systematically elucidated by Wellington (1957, 1960) with populations of tent caterpillars. Wellington was able to isolate four categories of tent caterpillar larvae (Malacosoma pluviale) on the basis of behavioral orientation to a light source. Type I larvae are active and orient well when isolated. Type IIa larvae are active and orient only in groups. Type IIb larvae are less active and disoriented in groups and as isolates. Type IIc larvae are sluggish and seldom develop. Various colonies of these larvae were constructed using varying percentages of each type and their behavior studied.

Wellington found that a colony consisting of more than 13% Type I larvae, more than 29% Type I and IIa larvae, and fewer than 21% Type IIc larvae were very active and constructed normal, elongated silk tents.

Comparisons of these laboratory colonies were made with field data on egg laying and dispersal into new areas and Wellington found that each larval type served a needed function in the colony. Type I larvae are capable of independent, directed movements even when isolated. On twigs and foliage they are capable of invading new territory unmarked by the silk trails of other larvae, although they will use such conditioning if it is present. Since they are the most active, Type I larvae are the first to respond to climatic changes, or any disturbing stimulus. Their movements stimulate some of the other larval types to follow them to new locations or on food foraging excursions. Wellington (1957) found that fed and vigorous Type I adults are largely responsible for relieving population pressure in the original foci by dispersing some of the next generation to more distant areas. Type I larvae exert a similar effect by dispersing the colony before parasite attacks become frequent. Type IIa larvae have a lower activity level, are incapable of independent orientation, and will travel a straight course in the presence of a Type I larva or a silk trail. They are of little value in extending the range of colony activities. However, they are a valuable component of a colony in that they help transmit "activity waves" throughout a resting colony, thus insuring that feeding intervals are not too long. Type IIb larvae are relatively inactive and depend for their survival on the more directed types of

larvae or on silk trails for finding food. It is this type of larvae that comprise the bulk of tent caterpillar colonies. Their presence insures the survival of at least some of the Type I and IIa larvae because (1) in tent construction they produce a denser, tougher mat of silk, since their inactivity causes silk strands to become concentrated (a denser tent prevents colony desiccation and predation), and (2) their presence helps the colony absorb losses due to predation or parasites, if these attacks are sporadic. Type IIc larvae are the weakest members of the colony and probably never contribute to the next generation. Their function in the colony is not well worked out, but it is detrimental if they are present in large numbers. Possibly they serve as the vanguard for picking up infections and diseases. All Type II larvae help subdue the activities of Type I larvae and keep the colony from becoming too active.

Comparisons of natural colonies or of artificial colonies made up of various densities of larvae and various percentages of larval types yielded similar results in that the make up of the colony largely determines its mortality rate and dispersal ability. More active colonies suffer many of their losses during the wandering of small groups led by Type I larvae. The most sluggish colonies are eliminated simply because they do not wander enough, but stay on their tents to starve or fall prey to disease.

Studies of natural colonies (Wellington 1960) revealed a cyclicity of density and types of colonies from 1956-1959. In each case Wellington found that the initial decrease in active colonies was a result of the deteriorating quality of the resident stock. That

is, the viable proportion of individuals changed in favor of more Type II larvae. Increases of active colonies was a result of the elimination of the less viable and moribund sluggish colonies from the population by extrinsic and intrinsic mortality factors. This increase occurred only after a period of decreasing density.

Wellington's results indicate that Chitty's (1960) hypothesis may be applicable to insect populations. Chitty (1960) hypothesized that populations are capable of limiting their own densities through deteriorations in quality that occur whether or not extrinsic mortality factors are operating, and that such reductions in vitality may lead to increasingly severe effects of some mortality factors, such as weather, upon the weakened population. This hypothesis assumes an "intrinsic weakness" within the population. The intrinsic weakness in the case of tent caterpillars is the Type II larvae, particularly Types IIb and c. The more active Type I larvae produce Type I adults which fly off to oviposit. The less active Type II larvae produce less active Type II adults which oviposit near their birthplace. This gradual buildup of large numbers of Type II larvae leads to sluggish, slowing, deteriorating colonies whose numbers far outweigh the few active colonies established by active adults. After an initial increase these colonies will die. Intermediate colonies will also die out when their more sluggish members succumb (i.e., to sudden weather changes) because they are not numerous enough or sluggish enough to produce the necessary tent silk and mass of insulating bodies.

There are strong indications from these studies with Lepidoptera that, exclusive of environmental factors, spatial distribution is dependent upon biological conditioning involving a preference on the part of the larvae, some sort of social behavior be it sex or animal-animal interactions, and the probability that such responses are age dependent. My studies strongly support this view. However, unlike the studies presented in this review, my studies have been approached from the point of view that we must first understand individual behavior before the myriad interactions within a population are explicable.

CHAPTER II

BEHAVIORAL AND LIFE HISTORY CONSIDERATIONS OF THE GREATER WAX MOTH, GALLERIA MELLONELLA (L.) (LEPIDOPTERA: GALLERIIDAE)

Behavior and Life History

There is a rapidly accumulating literature on Galleria mellonella, although it is a relatively new species to behavioral studies. Galleria mellonella (L.) is of biological interest because of its developmental patterns, food habits, ecological and behavioral adaptations, and general adaptability as an experimental organism. The larvae and adults have been used in studies of biochemical, nutritional, and digestive processes (Haydak 1936, 1940; Niemierko and Cepelewica 1950; Niemierko and Wlodawer 1950, 1952; Niemierko and Wojtezak 1950; Zielinska 1952; Przelecka 1956; Dadd 1964, 1966; Balazs 1958; Young 1961, 1964), physiology (Bell 1940; Wojtczak 1952a, b, and c; Pipa 1963; Edwards 1966; Harshbarger and Moore 1966; Alexander 1970), development (Paddock 1913; Andrews 1921; Chase 1921; Haydak 1940; Whitcomb 1942; Beck 1960), comparative arthropod anatomy (Borchert 1933; El-Sawaf 1950; Milum 1952a and b), and comparative ecology and behavior (Paddock 1913; Plath 1934; Milum and Geuther 1935; Haydak 1936; Milum 1952a and b; Dutky et al. 1962; Burkett 1962; Oertel 1962, 1963; Woolever and Pipa 1970).

The origin of Galleria mellonella is not well documented, but it is probably of wholly Oriental origin (Paddock 1913). This organism was even known to writers of antiquity. Aristotle tells of the injury they caused in bee hives. Today, however, Galleria has a world-wide distribution (Paddock 1930; Imms 1948; Skaife 1954) and was introduced into the United States sometime around 1805, shortly after the introduction of the honey bee (Apis mellifera).

Galleria mellonella (L.) is a parasite in honey bee (Apis mellifera) hives, the larvae feeding on the pollen, honey-laden brood combs, and possibly bee larvae. It is of economic importance since it is estimated that large losses to the honey bee industry are caused by this and related species (Whitcomb 1942), particularly to combs in storage. It is not yet clear how a bee hive first becomes infested with Galleria, but once infestation occurs the hive is eventually destroyed. There appears to be an indirect relationship between "strength" of a hive and Galleria's ability to invade it. Paddock (1913) states that if the bee colony has a vigorous queen and is kept strong, Galleria cannot enter the hive to deposit eggs. Oertel (1962) records a similar finding, and Whitcomb (1942) notes that weak, diseased, starved, or otherwise abnormal colonies are prey to the wax moth, and in such colonies the combs are entirely destroyed. However, wax moth injury is secondary since strong colonies will defend themselves well against attack.

It appears that Galleria (larvae) is chiefly dependent upon honey bee hives for food, but it has been reported to infest other hives as well. Oertel (1962) found Galleria to be a pest of the

stingless bees of Brazil, and further states that Walrecht (1958) found them in wasp nests (Vespa and Vespula). However, Plath (1934) and Sladen (1912) could find no evidence of such infestations in bumble bee nests. Oertel (1963), on the other hand, found that under certain conditions, bumble bee cells can serve as a host of Galleria. He further hypothesizes that perhaps bumble bee nests help to maintain wax moth populations in the absence of honey bee combs.

The life cycle of Galleria mellonella is typical of holometabolus insects, consisting of egg, larval, pupal, and adult stages. There are several overlapping generations per year in the wild, and over-wintering can occur as larva, pupa, or adult (Paddock 1913).

Each female moth lays hundreds of eggs in clusters. Paddock (1913) reports the eggs to be elliptical in shape, measuring 0.48 mm in length and 0.43 mm in width, and pearly white in color. Andrews (1921) says the eggs are spherical or pear-shaped, depending on where laid. Length of egg stage is variable, depending on temperature. Paddock (1913) reports the egg stage to last 10-12 days whereas Whitcomb's (1942) data indicates length of egg stage to be 5-8 days at 24-27°C. In our laboratory we found the egg stage to last 5 days at 32±1°C. Lowering the temperature will delay hatching.

When first hatched the larvae are white and about 3 mm long (Paddock 1913). Chase (1921) reports newly hatched larval length to be 2 mm or less, which we have found to be more accurate. The young larvae are extremely active, with prominent thoracic legs and the ability to move forward or backward with equal rapidity (Milum 1952). Older larvae exhibit a range of colors from whitish to dark

gray, are about 18 mm long, have a small head and large body, and have lost the rapid movements characteristic of young larvae. Older larvae are also round in shape, whereas young larvae are dorsal-ventrally flattened.

Paddock (1913) reports the length of larval life to be 35-40 days, and Chase (1921) and Beck (1960) state that larval development consists of several instars. Development of the larvae is heavily dependent on temperature, light, food source, and density. Accounts in the literature differ because these variables differed or were not specified, observations were taken in the field and the laboratory, and what constitutes larval development was not operationally defined. For example, the average duration of development (from hatching to emergence as adults) has been reported to be 29.3$\pm$ 1.5 days (Haydak 1940), 42-49 days (Metalnikov 1908), 90-120 days (Paddock 1913), 42-53 days (Andrews 1921), 33-54 days (Chase 1921), 62-64 days (Borchert 1933), and from 28 days to 4 or 5 months (Whitcomb 1942). In many of these cases the rearing conditions were not specified. Whitcomb (1942), however, reared eggs at 85-95°F, which is about the temperature normally found in an active bee hive. Milum (1952) agrees with this data, saying that the average larval period is 28.85 days at 35°C. In our laboratory, however, we found the average length of larval life (from hatching to cocoon spinning) to be 18-20 days at 32$\pm$1°C.

During the larval stage, larvae spin extensive "silken" tunnels from modified salivary glands and mouth parts. These tunnels are spun throughout their food source and become covered with frass

and food. It would appear that at no time during larval development do they leave the food or the tunnels.

Galleria larvae exhibit a rapid growth rate under optimal conditions and the size distribution between ages is extreme (Figure 4.1). Beck (1960) has demonstrated that, at 35°C and under continuous darkness, larval weight doubles daily during the first 10 days of life.

One or two days before beginning to spin a cocoon, the larvae enter the "wandering phase." During this phase, they leave the food, probably stop eating, and search for a pupation site. They continue to spin silk during the wandering phase but no longer in discrete tunnels. It has been hypothesized that the mechanism initiating this phase is a cessation of eating, but this has yet to be tested. The wandering phase lasts about two days, at the end of which time the larvae settle down and begin spinning a cocoon.

The larvae usually pupate in cracks and crevices in the hive walls although they may pupate in the refuse and webbing under the combs (Paddock 1913). Whitcomb (1942) observed cocoons in the refuse under the comb and in the mass of tunnels and refuse of wax on the hive frames. Once a suitable pupation site has been selected, the larvae spin a dense, tough silken cocoon, presumably utilizing similar behaviors as those described by Van der Kloot and Williams (1953a) for Cecropia, and pupate within this protective structure. Before changing into a pupa, however, three flap-like slits are cut in an exposed end of the cocoon to facilitate emergence as an adult (Milum 1952). The duration of the pupal stage ranges from 8 to 62 days, depending on temperature (Whitcomb 1942), after which time eclosion occurs and the

adult stage begins. Males eclose a few days before females, and are generally longer-lived.

The adults are small, 15 mm long moths with a wing span of 30-32 mm (Paddock 1913; Whitcomb 1942; Milum 1952) and are dark gray in color. The size and color of the adults is affected by the type of food consumed by the larvae and by the length of the developmental period (Whitcomb 1942).

There is probably no direct interaction between the adults and larval or pupal stages. This hypothesis is supported by several observations. Galleria adults die shortly after mating and have never been observed caring for either eggs or larvae. The adults lack mouth parts and do not eat (Oertel 1962). Their only function in life is reproduction and invasion of new bee hives.

Galleria adults exhibit a sexual dimorphism (Whitcomb 1946; Paddock 1913). Males are smaller than females, and the forewing of the male is deeply scalloped while that of the female is straight edged. The female also has two short, prominent pointed palps on the front of the head, whereas the male lacks these palps (Milum 1952).

Mating occurs at night (Paddock 1913), but several reports in the literature indicate mating occurs during the day as well. If maintained in constant darkness, mating occurred at all hours of the day in our laboratory. Once mated, a female lays anywhere from one to several hundred eggs in the cracks and crevices of the bee hive. There is, however, considerable conflict in the literature on just how many eggs a female lays. Paddock (1913) says 1018, Whitcomb (1942) says less than 300, and Milum and Geuther (1935) say an average

of 754 eggs per individual female. The stimulus for egg laying seems to be copulation with a male. Non-mated females, however, will eventually lay their eggs, which do not hatch.

How adults leave a hive to infest a new hive has not been investigated. Once they have left a hive, however, a new hive is probably invaded by the female laying her eggs in cracks and crevices on the outer walls of the hive. The newly hatched larvae could then work their way into the hive and reach the combs.

Spinning Behavior and Preferences

Galleria larvae exhibit three distinct spinning behaviors: (1) discrete larval tunnels, (2) diffuse webbing during the wandering phase, and (3) discrete cocoons in which pupation occurs. There are no studies dealing with these behaviors or with how the result of such behavior affects the behavior or preferences of the other larvae in the population. Such studies would be of value when one considers that the larval phase of most holometabolus insects is the longest phase and larval behavior could have significant effects on preceding and succeeding generations. For example, the size and color of the adults is a function of the degree of larval crowding and food source. Developmental sequence and mating might be affected by larval behavior and development. My studies with Galleria have shown that biological conditioning, of which spinning behavior is a part, functions in the spatial distribution of the larvae, and that larval preferences for conditioning are a function of age and number of larvae present.

By definition, Galleria larvae biologically condition their environment. Spinning behavior is an obvious physical conditioning, but there may also be chemical conditioning from secretions and excretions, such as frass. These biological conditionings, although not called such, have been alluded to in the literature. Milum (1952) found that newly hatched larvae feed on isolated portions of the comb, forming silken tunnels with added frass and bits of comb. They gradually assemble in a mass of webbing with tunnels extending through the remainder of the combs in search of food.

Several functions for larval spinning behavior have been postulated, but never tested. Paddock (1913) says that the only time the larvae are at the mercy of the bees is just after they hatch and are trying to chew into the comb. Once they chew into the comb they make tunnels directed toward the bottom of the cells. These tunnels afford protection and food for the larvae and also lead to their desired feeding place, the center of the comb. When the center of the comb is reached, the larvae leave their tunnels and wander over the bottom of the cells or tunnel along the midrib from cell to cell. If disturbed, they seek their tunnels for protection. Eventually a massive silken gallery has been spun which gives almost complete protection from the bees. From this central gallery feeding is extended out along the bottoms of the cells or the middle of the comb. The silk is spun wherever the larvae go so that soon the bottoms of the cells are replaced by a layer of silk threads covered with excrement and particles of chewed wax. As outward feeding continues the threads of silk are extended to cover the new feeding grounds and not only

serve to protect the larvae, but also act as a scaffold to support the damaged cells. Therefore, the feeding area gradually extends from the point of infestation to the entire comb, and, if the comb has insufficient food, they feed in the refuse beneath the comb. These feeding larvae consist of overlapping generations and all stages are found feeding and spinning at any one time.

These observations suggest that larval spinning functions as (1) protection from the bees, (2) scaffolding to support damaged cells, and (3) a mechanism to direct feeding behavior. Implicit in these observations is the idea that the silk tunnels may be involved in the spatial distribution of the larvae. The fact that all larval ages are present at any one time also raises the question as to what interactions might be occurring and whether there is preferential use of the silk galleries according to age.

The hypothesis that larval tunnels serve as protection from the bees is particularly interesting in light of Free's (1961) article on the stimuli releasing the stinging response of honey bees. Free discovered that bees preferentially sting dark colors, rough surfaces and rapidly moving objects. Galleria larvae move in rapid, jerky motions, but in their light, smooth tunnels their movements may not be detected.

There is no literature on spinning behavior or preferences during the wandering phase. The assumption appears to have been made that a wandering larva is oblivious to all else during this phase except finding a suitable pupation site. The mechanism(s) for the

transition in spinning behavior from tunnels to webs and from webs to cocoons has not been investigated.

There are several studies dealing with cocoon spinning behavior in Lepidoptera. Van der Kloot and Williams (1953a and b) discuss the role of the internal and external environments in relation to cocoon spinning in Cecropia. Certain preferences were found in relation to location and position of the cocoon. However, the initiation of spinning does not invariably depend upon the presence of an external environment which would allow the construction of a normal cocoon. Caterpillars will spin in environments where it is impossible for the organism to construct more than a single sheet of silk. The longer a silkworm is kept in an inadequate environment, the greater the likelihood of its beginning to spin. It appears that there is an increasing internal factor, acting as a stimulus for spinning, which lowers the threshold for external stimuli to a level where the larva will spin in a normally inadequate environment. Van der Kloot and Williams' experimental results also emphasize the importance of tactile and proprioceptive stimuli in cocoon spinning.

Some data on Galleria demonstrate preferences for cocoon sites. Whitcomb (1942) records that when larvae spin cocoons they firmly attach themselves to the sides of the hive, the frame, or some such solid object. Some larvae, however, prefer tunnels and hive debris as cocoon sites. Woolever and Pipa (1970) experimentally investigated the spatial and feeding requirements for pupation of last-instar larvae. They discovered that emergence of pupa from LSU's (the first stage of cocoon spinning) can be significantly delayed by

depriving the larvae of "sufficient" free space. Last-instar larvae also require a minimum of two days' exposure to standard diet before metamorphosis will occur.

Anything affecting the natality, mortality, or dispersion of the larvae will have consequences to the species as a whole. Since Galleria is a young "society," that is there are no adult-young interactions from which the larvae might behaviorally learn, it would be advantageous for the larvae to have the ability to pick and choose those biotic and abiotic cues best suited for its development and success as a species. There are indications of this ability in the literature, particularly with reference to variables affecting developmental time, natality, and mortality. My studies indicate that the relationship between Galleria's preferences for biological conditioning and dispersion is a close one.

Food Preferences

Certain areas in a bee hive have better food sources than others. Older hive combs are often deserted by the bees and not maintained, whereas areas in which the bees are actively rearing their own larvae will be rich in honey and pollen. In an already infested hive, some areas will have been conditioned by previous Galleria generations, while others will not. Such conditioned areas may (1) indicate an area already well worked over or heavily populated, or (2) such areas may signal an immediately usable food source accompanied by immediate protection. There are indications in the literature that Galleria larvae exhibit preferences in selecting a food source on which to develop.

Beck (1960) reported on an artificial dietary medium for laboratory rearing of Galleria mellonella larvae which yielded optimum growth rate, as measured by the number of instars. It had earlier been reported that larvae are capable of utilizing beeswax as a source of dietary lipids (Dickman 1933; Waterhouse 1959). Yet Haydak (1936, 1940) found beeswax not to be a required nutrient, since growth and maturation occurs in its absence. This appears to be true, but Beck (1960) notes that if beeswax is included in the diet, larval growth is significantly improved. Beeswax also has an effect on the physical consistency of the medium, rendering the diet somewhat less compressible. I found that larvae avoided diets lacking wax. However, it was not determined if this avoidance was due to some missing nutrient or to the physical consistency. Paraffin has a similar physical effect, but less of a stimulating effect on growth. Since paraffin is indigestible, its stimulating effect was attributed entirely to the altered physical properties of the diet, and the better growth on beeswax diets was attributed to some added nutrient (Beck 1960). Young (1961) found that beeswax and some fatty acids enhance larval growth. Niemerko and Wlodawer (1950) reported that the excreta of the larvae contains 60-65% fatty acids. This percentage is similar to that found in beeswax, an indication that the larvae eat the wax. However, undigested wax is a major part of the fecal matter.

Whitcomb (1946) felt that larvae get nourishment from impurities in the wax. Chase (1921) found that many of these impurities are from bee and Galleria frass, dead moths, and various larval casts. Much earlier, Braun (1867) maintained that wax moth larvae

devour combs in order to obtain pollen and other nitrogenous substances, such as cocoons and excreta. Most of this research on larval nutrition lack tests of actual larval preferences for various diets. Chase (1921), however, experimented with several food sources. The one she found to be most successful was a mixture of chopped-up comb wax, bee bread, soft dead moths, and honey kneaded into a small loaf from which slices were cut. Newly hatched larvae first selected for food the dead moths, and as development proceeded their preference switched to the comb. Andrews (1921) found that larvae could not be raised solely on dead moths, which may account for the switch in preference noted by Chase.

Haydak (1936) hypothesized that Galleria larvae are seeking pollen or other materials and that burrowing in wax is really a secondary, acquired characteristic which serves as self-protection. However, his experiments did nothing to shed any light on this hypothesis. He raised larvae on several diets, but only in those diets containing dried yeast did the larvae produce large quantities of silk. Growth was also greatest on yeast diets. Larvae reared on non-yeast diets produced very little silk and covered their sparse tunnels and cocoons with frass. (It is interesting to note that the lesser wax moth, Achroia grisella, normally spins only sparse tunnels and exhibits the behavior of covering these tunnels with frass and other debris.) The low silk production on deficient diets may be a function of insufficient amino acids essential for silk production.

The quantity and quality of larval food affects certain adult morphological characteristics. Slowly developing larvae, as a result

of poor nourishment, low temperatures, or high humidity, transform into small adults. Larvae fed on dark brood combs transform into adults that are dark gray or almost black. Larvae reared on other deficient sources have been recorded to produce small adults ranging from silvery-white to gray in color, depending on the food source (Paddock 1946). We noted in our laboratory that overcrowded larval colonies are slow to develop and produce tiny adults. This is probably a function of the food supply becoming deficient, or might also be related to substances being added to the medium by the crowded larvae.

The larvae of Galleria mellonella (the greater wax moth) and Achroia grisella (the lesser wax moth) both infest honey bee hives, although they occupy ecologically dissimilar niches. The larvae of these two species are virtually indistinguishable. Milum and Geuther (1935) stumbled upon a possible method for distinguishing between these species, based on food preferences. Young Galleria larvae will develop on brood comb, but not on comb honey. Older larvae will develop on both. Achroia larvae appear to develop on almost anything. Although not so stated, it would appear that Galleria will preferentially select brood comb when in its early instars.

Rearing and Maintenance of Stock Cultures

Stock cultures of the greater wax moth larvae were maintained in 150x75 mm crystallizing dishes. The dishes were covered with a circular piece of plexiglass with a 15 mm hole in the center. The plexiglass cover was held in place with two pieces of masking tape, one on each side, and the center hole was covered with tape. This

rearing container was originally patterned after a similar one used by Beck (1960). Beck, however, plugged the central hole with cotton and held a glass top on with a layer of vaseline. These procedures proved rather cumbersome. The vaseline layer was intended to prevent larvae from escaping. However, behaviorally, larvae exhibit a downward movement, and since the vaseline melts in an incubator, I found masking tape to be more efficient. The cotton plug used by Beck was for ventilation. However, I found that when pupation occurred the larvae either pupated in this plug or easily escaped through the hole. Since sufficient ventilation occurred between the plexiglass top and the crystallizing dish, the central hole was covered with masking tape. This arrangement also prevented water condensation from forming inside the dish.

About 200 grams of dietary medium was added in a loose layer to each dish. Larvae were then added, the top secured, and each dish placed in an incubator. The larvae were raised in total darkness at 32 ± 1°C and 75% R.H. (Egg, larval, pupal, and adult stages were all reared in incubators under identical conditions).

The dietary medium (Table 2.1) was a 3%, by weight, wax diet based on the diet used by Beck (1960). This diet was prepared as follows: The beeswax was melted on a small hotplate. While the wax was melting, the pablum and yeast were weighed and mixed together. Honey, water, and glycerin were weighed as a unit, added to the pablum and yeast, and thoroughly mixed. The melted beeswax was then mixed in. This mixture was usually made in 1000 gram quantities and then equally divided among five crystallizing dishes for colony use.

Table 2.1.

Artificial Dietary Medium for Laboratory Rearing of the Greater Wax Moth, Galleria mellonella (L.)

Constituent	Amount (G.)
Honey	24.25
Glycerin (U.S.P.)	21.34
Water	8.73
Pablum, mixed cereal	32.98
Active Dry Yeast	9.7
Beeswax (with impurities)	3.0
	100.00

The colony was maintained as follows: Adults were put in a 5 gallon fish tank with a plexiglass top. Several pieces of wax-coated weighing paper were folded, accordion-fashion, and placed in the tank. Females laid their eggs on these papers which could then be retrieved at will. As new adults eclosed in the colony they were added to the tank so as to maintain a constantly breeding colony. To remove the eggs, the breeding tank was placed in a refrigerator for five minutes. This slowed adult activity so the top could be removed in order to retrieve the eggs without the adults escaping. The addition of adults was also done at this time. These procedures were performed at 8 AM every day, and the breeding tank was then returned to the incubator with fresh egg papers.

The eggs were cut off of the paper and put in a 100x15 mm plastic petri dish. These dishes consisted of two petri dish bottoms taped one on top of the other. This was done to allow extra space for large egg lays and to prevent newly hatched larvae from escaping. These egg dishes were devoid of food so that newly hatched larvae

could be seen and time of hatching easily determined. Newly hatched larvae are too tiny to be found in food, which would make hatching time impossible to calculate. Hatching time was needed to determine future larval ages and to synchronize the colony. Once the eggs had been cut off the paper and put in these dishes, the dishes were placed in the incubator.

As soon as eggs began hatching, the contents of the petri dish were put in a crystallizing dish containing food, and left-over eggs were removed 24 hours later. Due to this procedure, the larvae in any particular crystallizing dish consisted of larvae hatched in a 24 hour period of time. For example, one-day-old larvae are actually 1 day old $\pm$ 12 hours, and so on for all ages. The number of larvae in any one dish was variable, depending on the size of the hatch.

When larvae in the crystallizing dishes entered the wandering phase at about 16 days of age, as indicated by their tendency to move upward in the dish, they were culled. About 100 larvae were removed and placed in a new crystallizing dish with fresh food, where pupation occurred. Pupation appeared to last 7-9 days, although this was not precisely measured. This procedure was followed because it was found that adult eclosion was delayed if the culture dishes in which the larvae pupated were crowded.

When the adults in the new crystallizing dishes emerged they were put in the breeding tank and the cycle continued.

The initial larval stocks of Galleria were obtained from a local fish bait wholesaler, Victor A. Wilicht of Bath, Michigan. In an attempt to keep inbreeding at a minimum, fresh stocks of 500 larvae

were purchased from this dealer once a month and mixed with our established colony. Of course, all animals were from the same original colony so some inbreeding occurred. However, since hundreds of adults were being bred at any one time, inbreeding was probably at a minimum. As a check on whether these procedures were resulting in behavioral changes, periodic behavioral tests (controls) were run. These tests consisted of asking whether larval responses to biological conditioning changed with time. The results showed that behavioral responses to conditioning remained constant. These tests are discussed in Chapter IV.

CHAPTER III

HOTELLING'S T^2 STATISTIC FOR MULTIVARIATE ANALYSIS: A "NEW" APPROACH TO LONGITUDINAL STUDIES AND SERIALLY CORRELATED DATA

Introduction

Scientists have long been perplexed by the appropriate means to analyze phenomenon in behavioral development, the major problem being how to analyze developmental data and satisfy the assumption of independence. Classically two kinds of experiments have been used in developmental studies. One is the cross-sectional experiment which measures different individuals at each age or time period. Although this approach meets the assumption of independence of measurements, it is not an appropriate method for analyzing individual behavioral changes over time. It also often necessitates a small sample size at each time point, particularly if the time series is a long one. The second approach is the longitudinal study in which an individual is repeatedly measured throughout time, with the result that each succeeding measurement incorporates the preceding ones. This is an appropriate procedure for looking at changes in individual behavior over time but may violate the assumption of non-serially correlated data. It is the longitudinal study which is of concern in this paper.

Biologists seem well versed in statistics when it comes to considering the assumption of normality and homogeneity of variances

in their statistical procedures, but serial correlation is another matter. To avoid violating the assumption of independence of the error terms underly-all parametric statistics, biologists have typically done one of the following with longitudinal data:

(1) They ignore the statistical assumptions and analyze their data as if measurements had been independently collected. As Gill and Hafs (1971) point out, such a procedure leads to misinterpretation of results because of correlated error terms.

(2) Often a "grand mean" is calculated from the raw data and analyses are carried out on these means. This procedure eliminates the problem of independence but it also throws away much valuable data. It also may lead to incorrect conclusions since the analysis may conclude that two groups are not different on the basis of their means, when in fact the curves for each group are very different.

(3) Biologists often circumvent the assumptions in parametric statistics, except for independence, by resorting to non-parametric analyses. However, most non-parametric statistics are not very powerful or sensitive.

The objective of this paper is to present a statistical procedure for the analysis of developmental data by treating time as a multidimensional random variable and discussing the statistical considerations necessary before drawing inferences from such data. This will be done by first discussing the test statistic and then the assumptions underlying it and how these assumptions may be tested.

The method to be presented is also valid for conventional multivariate analyses drawn from cross-sectional data.

In 1931, Hotelling presented a generalization of the familiar univariate Student's t-test on means of normal populations to tests involving the mean vector of responses drawn from the multivariate normal population. Hotelling's procedure was originally intended for situations in which one wishes to ascertain whether two samples of organisms with p measurable characteristics arose from the same population. For example, one may wish to ascertain if body weight, ear length, and tail length are the same in two populations of mice given different treatments. Such data may be analyzed by comparing each group to an expected mean vector or by comparing the groups to each other. These procedures have come to be known as the "One" and "Two" Sample Hotelling's T^2 Statistics for Multivariate Analysis, respectively, and both utilize a matrix algebra approach to their solutions.

For developmental data, however, the time scale is of interest. By modifying Hotelling's procedures such an analysis is possible. The modification consists of replacing the "p measurable characteristics" with "t measurable time units" and treating time as a multidimensional variate. By coupling this modification with the Durbin-Watson test for serial correlation and Box's Test for heterogeneous variances, all of the assumptions of a Multivariate Analysis of Variance may be tested.

Morrison (1967) presents a detailed discussion of Hotelling's T^2 procedures from which the formulas in this paper were taken, but the basics will be presented here, particularly as they relate to the

time modification. However, Morrison does not deal with the assumptions underlying this test and these will be thoroughly discussed. The procedure for the time modification was first brought to my attention by Dr. James Asher who utilized such an approach in a study of parthenogenesis and genetic variability (Asher 1970).

The nomenclature in this paper will consist of the following: Any letter with a wavy line under it refers to a matrix, i.e., $\underset{\sim}{A}$ or $\underset{\sim}{a}$. Entries within a matrix will be designated with lower case, subscripted letters, such as x_{ij} for the ith row and jth column of $\underset{\sim}{x}$. The number of rows in a matrix will be designated as N, which during the current discussion will also be the number of replications. The number of columns in a matrix will be designated as t, which is also the number of time units.

Hotelling's One Sample T^2 Statistic

The procedure for the one sample test is presented in flow chart form in Figure 3.1.

Let the response variate $\underset{\sim}{X}$ consist of t components distributed according to the multinormal low with mean vector $\underset{\sim}{\mu}$ and nonsingular covariance matrix $\underset{\sim}{\Sigma}$. The null hypothesis to be tested is:

$$H_o: \begin{bmatrix} \mu_1 \\ \cdot \\ \cdot \\ \cdot \\ \mu_t \end{bmatrix} = \begin{bmatrix} \mu_{o1} \\ \cdot \\ \cdot \\ \cdot \\ \mu_{ot} \end{bmatrix} \quad \text{and its alternative is: } H_1 \begin{bmatrix} \mu_1 \\ \cdot \\ \cdot \\ \cdot \\ \mu_t \end{bmatrix} \neq \begin{bmatrix} \mu_{o1} \\ \cdot \\ \cdot \\ \cdot \\ \mu_{ot} \end{bmatrix}$$

In practice the population mean vector $\underset{\sim}{\mu}$ and the entries in the population variance-covariance matrix are unknown.

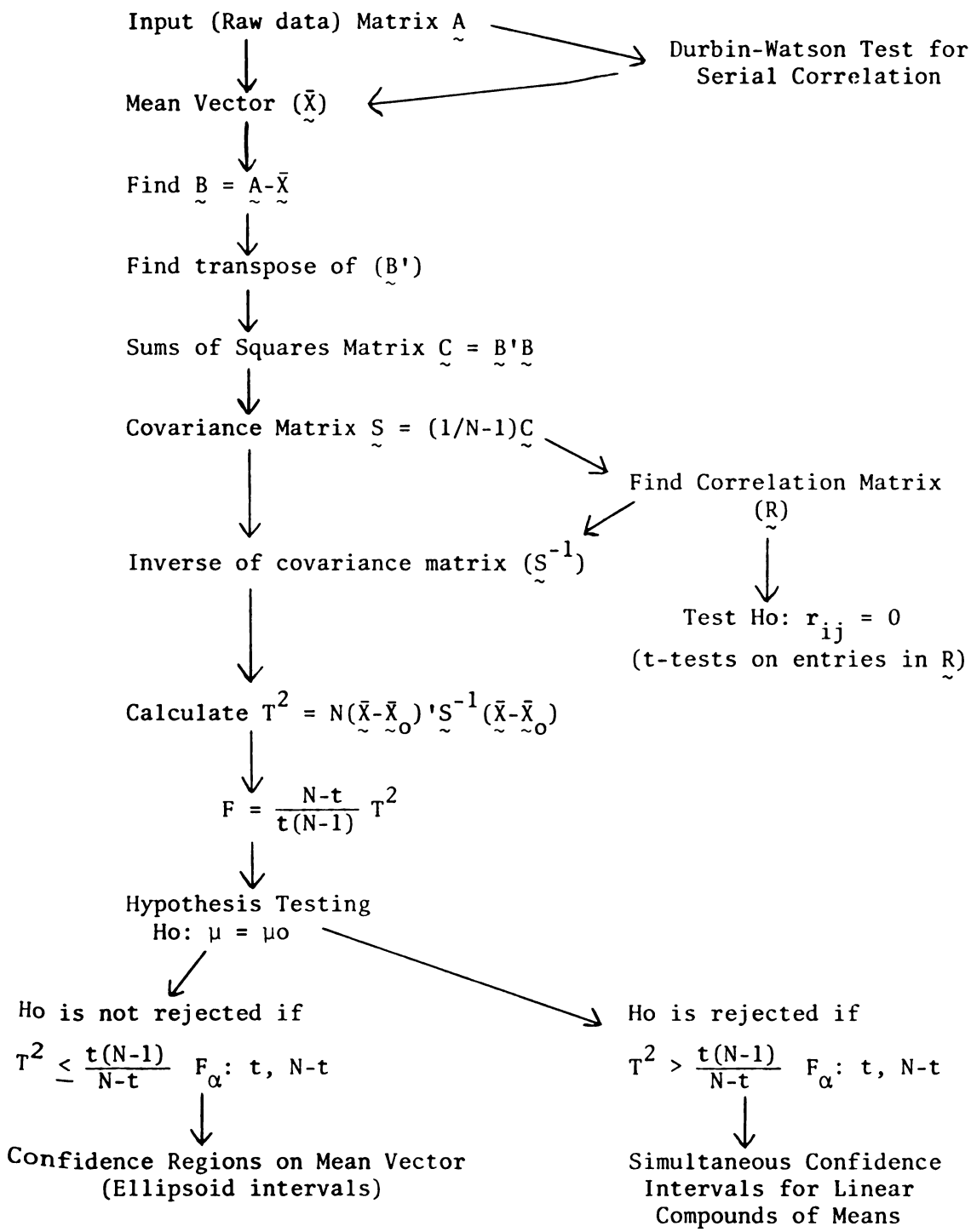


Fig. 3.1
Hotelling's One Sample T^2 Statistic.

The calculations necessary to test the null hypothesis are derived from a matrix of N independent observation vectors and t time units. Let $\underset{\sim}{A}$ be such a raw data matrix, such that

$$\underset{\sim}{A} = \begin{array}{c} \\ 1 \\ \\ \\ N \end{array} \begin{array}{c} \begin{array}{ccc} 1 & \cdots & t \end{array} \\ \begin{bmatrix} a_{11} & \cdots & a_{1t} \\ \vdots & & \vdots \\ a_{N1} & \cdots & a_{Nt} \end{bmatrix} \end{array}$$

and each entry in the matrix is some measurable characteristic of interest over time. It is necessary that $N > t$ since the degrees of freedom for the test statistic are t and N-t, and if $N \leq t$ this test cannot be made.

From this matrix are calculated the $\underset{\sim}{\bar{X}}$, $\underset{\sim}{B}$, $\underset{\sim}{B}'$, $\underset{\sim}{C}$, $\underset{\sim}{S}$, and $\underset{\sim}{S}^{-1}$ matrices, where

$\underset{\sim}{\bar{X}}$ = the mean vector whose entries are the means calculated from each column in $\underset{\sim}{A}$. For example,

$\underset{\sim}{\bar{X}}_1 = [\bar{a}_1, \bar{a}_2, \quad \cdots \quad \bar{a}_t] \; / \; N$

$\underset{\sim}{B}$ = the matrix resulting when each entry in $\underset{\sim}{\bar{X}}$ has been subtracted from each entry in the corresponding columns of $\underset{\sim}{A}$.

$$\underset{\sim}{B} = \begin{array}{c} \\ 1 \\ \\ \\ N \end{array} \begin{array}{c} \begin{array}{ccc} 1 & \cdots & t \end{array} \\ \begin{bmatrix} a_{11}-\bar{a}_1 & \cdots & a_{1t}-\bar{a}_t \\ \vdots & & \vdots \\ a_{N1}-\bar{a}_1 & \cdots & a_{Nt}-\bar{a}_t \end{bmatrix} \end{array}$$

$\underset{\sim}{B}'$ = the transpose of $\underset{\sim}{B}$

$\underset{\sim}{C}$ = the sums of squares and cross-products matrix calculated as $\underset{\sim}{B}'\underset{\sim}{B}$

$\underset{\sim}{S}$ = the variance-covariance matrix calculated as $\underset{\sim}{C}/N-1$

$\underset{\sim}{S}^{-1}$ = the inverse of $\underset{\sim}{S}$

For the one sample test it is necessary to be able to determine, a priori, an expected mean vector ($\bar{\underset{\sim}{X}}_o$) against which the calculated mean vector ($\bar{\underset{\sim}{X}}$) is to be compared.

The test statistic is

$$T^2 = N(\bar{\underset{\sim}{X}}-\bar{\underset{\sim}{X}}_o)' \underset{\sim}{S}^{-1}(\bar{\underset{\sim}{X}}-\bar{\underset{\sim}{X}}_o).$$

When the null hypothesis is true, the quantity

$$F = \frac{N-t}{t(N-1)} T^2$$

has the F distribution with degrees of freedom t and N-t.

$$\text{If } T^2 \leq \frac{t(N-1)}{N-t} F_{\alpha,\ t,\ N-t}, \text{ Ho: } \bar{\underset{\sim}{X}} = \bar{\underset{\sim}{X}}_o.$$

$$\text{If } T^2 > \frac{t(N-1)}{N-t} F_{\alpha;\ t,\ N-t}, \text{ Ho: } \bar{\underset{\sim}{X}} \neq \bar{\underset{\sim}{X}}_o.$$

If the null hypothesis is not rejected, it is possible to calculate a confidence region for the mean vector as follows:

$$N(\bar{\underset{\sim}{X}}-\bar{\underset{\sim}{X}}_o)'\underset{\sim}{S}^{-1}(\bar{\underset{\sim}{X}}-\bar{\underset{\sim}{X}}_o) \leq \frac{(N-1)t}{N-t} F_{\alpha;\ t,\ N-t}$$

This confidence region is represented by a single number which T^2 would have to be bigger than before the null hypothesis could be rejected. This value will change as N and t fluctuate, or as the α-level is varied.

If the null hypothesis is rejected, simultaneous confidence intervals for linear compounds of means are calculated as follows:

$$\underset{\sim}{a}'\bar{\underset{\sim}{X}} - \sqrt{\frac{1}{N}\underset{\sim}{a}'\underset{\sim\sim}{S}\underset{\sim}{a}\left[\frac{(N-1)t}{N-t}F_{\alpha;\ t,\ N-t}\right]} \leq \underset{\sim}{a}'\underset{\sim}{\mu} \leq \underset{\sim}{a}'\bar{\underset{\sim}{X}} + \sqrt{\frac{1}{N}\underset{\sim}{a}'\underset{\sim\sim}{S}\underset{\sim}{a}\left[\frac{(N-1)t}{N-t}F_{\alpha;\ t,\ N-t}\right]}$$

where, $\underset{\sim}{a}$ = a vector the experimenter specifies, and

$$\frac{(N-1)t}{N-t}F_{\alpha;\ t,\ N-t} = T^2_{\alpha;\ t,\ N-t}.$$

For example, if we wanted to look at the confidence interval around entry $\bar{a}_1$ of the mean vector, then $\underset{\sim}{a}$ = [1 0 0 . . . 0]. Such a procedure enables us to determine which of the responses have probably led to the rejection of the hypothesis on the mean vector. The probability that all such intervals generated by different choices of $\underset{\sim}{a}$ are simultaneously true is 1-α (Morrison p. 121).

If one wishes to determine whether the means are correlated, a correlation matrix may be calculated from the variance-covariance matrix. This is done by pre- and post-multiplying the variance-covariance by a diagonal matrix whose entries are $1/\sqrt{0^2}$ along the diagonal and zeros on the off-diagonal positions. The resultant matrix is the correlation matrix with ones on the diagonal and correlation coefficients on the off-diagonals. Each off-diagonal element can then be tested for significant correlation by calculating $S_{r_{ij}} = \sqrt{(1-r_{ij}^2) / (N-2)}$ (see Sokal and Rohlf 1969, p. 516) and comparing the result with an expected calculated value from Table Y of Rohlf and Sokal (1969).

Example 3.1.--An example will help clarify the single-sample techniques. Ten isolate larvae were given a two choice situation for three days, the choices being between a biologically conditioned and an unconditioned food lump. If the conditioned lump was preferred a 1 was recorded, otherwise a zero was recorded. The results are to be tested against an expected vector whose entries are all 0.5, assuming a random response.

The raw data matrix ($\underset{\sim}{A}$) is

$$\underset{\sim}{A} = \begin{array}{c} \\ 1 \\ \\ \\ . \\ . \\ . \\ \\ \\ \\ 10 \end{array} \begin{array}{c} \begin{array}{ccc} t_1 & t_2 & t_3 \end{array} \\ \begin{bmatrix} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 0 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 0 \\ 1 & 1 & 1 \\ 0 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \end{bmatrix} \end{array}$$

The sample variance-covariance matrix of responses is

$$\underset{\sim}{S} = \begin{bmatrix} 0.100 & -0.011 & -0.011 \\ -0.011 & 0.100 & -0.011 \\ -0.011 & -0.011 & 0.100 \end{bmatrix}$$

We wish to test, at the $\alpha = 0.05$ level, the null hypothesis that the observations came from a population with mean vector

$$\underset{\sim}{\bar{X}}_o = [0.5 \quad 0.5 \quad 0.5].$$

The quantity $(\underset{\sim}{\bar{X}} - \underset{\sim}{\bar{X}}_o) = [0.4 \quad 0.4 \quad 0.4]$.

The test statistic is

$$T^2 = [0.4 \quad 0.4 \quad 0.4] \begin{bmatrix} 10.2795 & 1.2705 & 1.2705 \\ 1.2705 & 10.2795 & 1.2705 \\ 1.2705 & 1.2705 & 10.2795 \end{bmatrix} \begin{bmatrix} 0.4 \\ 0.4 \\ 0.4 \end{bmatrix}$$

$$= 61.714286, \text{ and}$$

$$F = \frac{(10-3)}{3(10-1)} \quad (61.714286)$$

$$= 15.9994, \text{ and d.f.} = 3, 7.$$

Since this is a two-tailed test, the tabulated critical value of $F_{\alpha/2;\ 3,\ 7} = 5.89$. Since the observed F is far in excess of the tabulated F, we conclude that the null hypothesis is untenable and that the responses arose from a population of larvae with considerably higher responses to conditioning.

Since the null hypothesis has been rejected we would want to look at the simultaneous confidence intervals for linear compounds of the means. Let us calculate one such interval for the second mean of the calculated mean vector $\underset{\sim}{\bar{X}} = [0.9 \quad 0.9 \quad 0.9]$.

Let $\underset{\sim}{a} = [0 \quad 1 \quad 0]$.

Then,

$$\sqrt{\frac{1}{N} a'Sa \left[\frac{(N-1)t}{N-t} F_{\alpha/2;\ t,\ N-t}\right]} = 0.4766$$

and the interval for the second mean is

$$0.4234 \leq 0.9 \leq 1.3766.$$

This result says that if we rejected H_o because of a deviation in the second mean then any mean would have to be outside of this interval to be considered different. By regulating the entries in vector a we can set the variance attributable to any mean and ask similar sets of questions.

If the null hypothesis had not been rejected we would have calculated a confidence interval for the mean vector.

Then,

$$N(\underset{\sim}{\bar{X}}-\underset{\sim}{\bar{X}}_o)'\underset{\sim}{S}^{-1}(\underset{\sim}{\bar{X}}-\underset{\sim}{\bar{X}}_o) \leq \frac{(10-1)3}{10-3} F_{\alpha/2;\ 3,\ 7} \leq 22.7183.$$

Therefore, if $T^2 \leq 22.7183$ the null hypothesis is not rejected. Since our calculated $T^2 = 61.714286$, we rejected the null hypothesis.

From the variance-covariance matrix we can also calculate the correlation matrix which turns out to be

$$\underset{\sim}{R} = \begin{bmatrix} 1 & -0.111 & -0.111 \\ & 1 & -0.111 \\ & & 1 \end{bmatrix}$$

From the correlation matrix a matrix can be calculated to test whether the observed correlations are significant. This is done by calculating $S_{r_{ij}} = \sqrt{(1-r_{ij}^2) / (n-2)}$ for each of the off-diagonal entries and comparing that value with Table Y. For example, let us look at entry r_{12} of $\underset{\sim}{R}$.

$$S_{r_{12}} = \sqrt{[1-(-0.111)^2]/(10-2)} \text{ or}$$

$$= 0.3514.$$

From Rohlf and Sokal's (1969) Table Y we find that with one independent variable and d.f. = 8 a critical value of 0.632 at the $\alpha = 0.05$ level. Since our calculated value is less than the critical value we cannot reject the null hypothesis. In other words, the means are not correlated.

Hotelling's Two Sample T^2 Statistic

Hotelling's Two Sample T^2 Statistic (Figure 3.2) is an elaboration of the one sample test. Two response mean vectors are compared to each other, rather than comparing a mean vector with an expected vector. Two raw data matrices are required, both of which have measurable time units. The test statistic, however, utilizes a pooled variance-covariance matrix derived from the two sums of squares and cross products matrices.

Suppose that two independent random samples of observations on a multidimensional variate (i.e., time) have been obtained from two experimental treatments. It is to be assumed that the variates have a multivariate normal distribution with the same, although unknown, covariance matrix $\underset{\sim}{\Sigma}$ of rank t. However, the distributions may not necessarily have the same location parameters and we desire a test of the null hypothesis that the population mean vectors are equal,

$$H_o: \underset{\sim}{\mu}_1 = \underset{\sim}{\mu}_2$$

as opposed to the alternative hypothesis

$$H_1: \underset{\sim}{\mu}_1 \neq \underset{\sim}{\mu}_2$$

The mean vectors and sums of squares and cross products matrices are computed from their respective raw data matrices and a pooled variance-covariance matrix is calculated. The following table, taken from Morrison (1967, p. 125), summarizes these procedures.

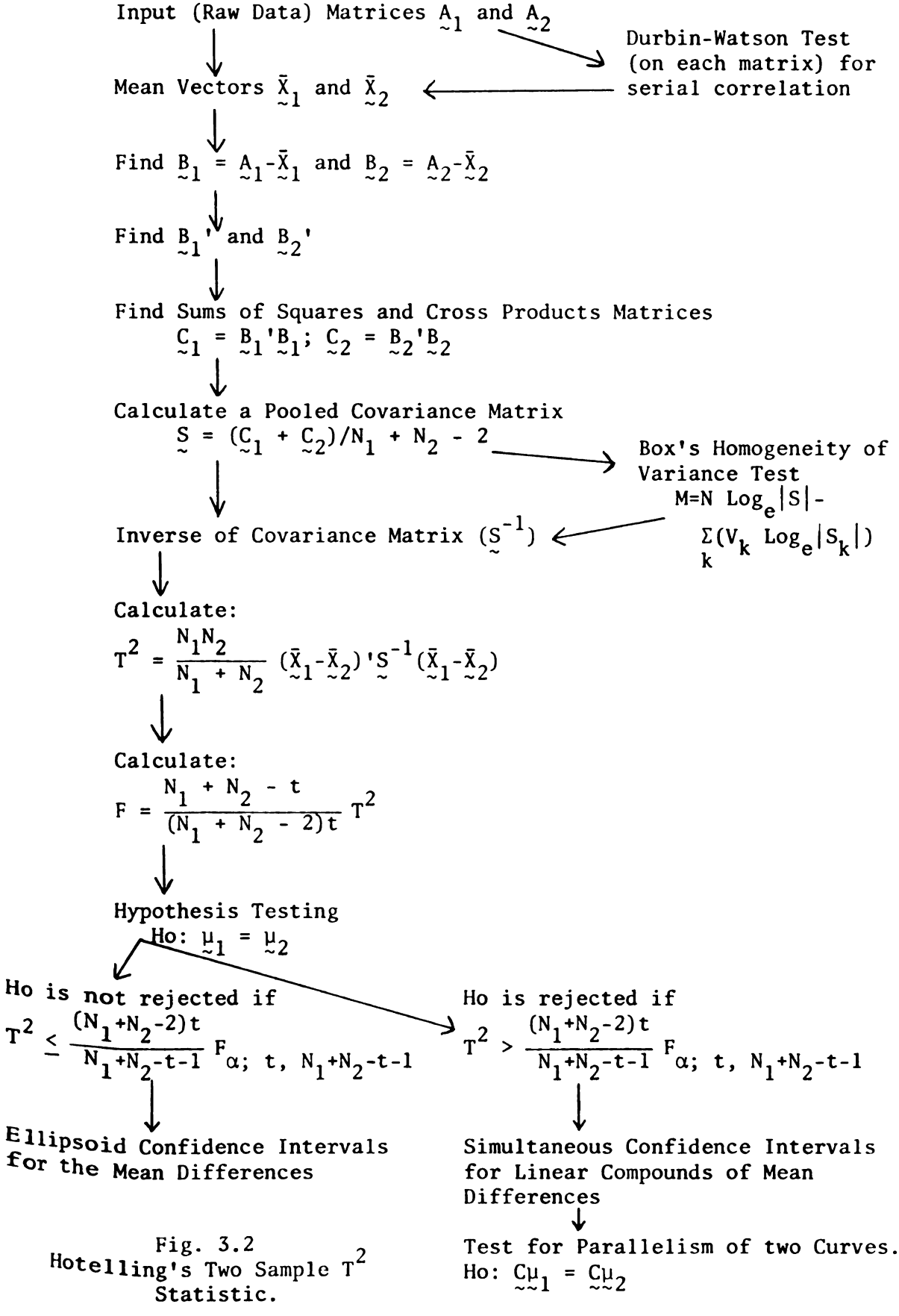


Fig. 3.2
Hotelling's Two Sample T^2 Statistic.

Table 3.1

Components of the Two Sample T^2 Statistic

	Sample 1	Sample 2
Sample size	N_1	N_2
Mean vector	$\bar{X}_1=[\bar{x}_{11}, \ldots \bar{x}_{1t}]$	$\bar{X}_2=[\bar{x}_{21}, \ldots \bar{x}_{2t}]$
Matrix of sums of squares & products	$\underset{\sim}{C}_1$	$\underset{\sim}{C}_2$
Pooled covariance matrix	$\underset{\sim}{S} = \frac{1}{N_1 + N_2 - 2} (\underset{\sim}{C}_1 + \underset{\sim}{C}_2)$	

The test statistic is

$$T^2 = \frac{N_1 N_2}{N_1 + N_2} (\bar{\underset{\sim}{X}}_1 - \bar{\underset{\sim}{X}}_2)' \underset{\sim}{S}^{-1} (\bar{\underset{\sim}{X}}_1 - \bar{\underset{\sim}{X}}_2)$$

and the quantity

$$F = \frac{N_1 + N_2 - t - 1}{(N_1 + N_2 - 2)t} T^2$$

has the F distribution with degrees of freedom t and $N_1 + N_2 - t - 1$.

It is important to note that the two raw data matrices being compared must be of equal dimension, that is $N_1 = N_2$ and $t_1 = t_2$. If the dimensions are unequal it becomes impossible to determine the degrees of freedom for the test statistic, or to obtain the pooled covariance matrix. If $T^2 \leq \frac{(N_1 + N_2 - 2)t}{N_1 + N_2 - t - 1} F_{\alpha;\, t,\, N_1 + N_2 - t - 1}$, the null hypothesis is not rejected, and the confidence region for the mean difference is

$$(\bar{X}_1-\bar{X}_2-\delta)'S^{-1}(\bar{X}_1-\bar{X}_2-\delta) \leq \frac{N_1+N_2}{N_1N_2}\left[\frac{(N_1+N_2-2)t}{N_1+N_2-t-1}\right]F_{\alpha;\ t,\ N_1+N_2-t-1},$$

where δ = the population mean of $(\bar{X}_1-\bar{X}_2)$. If

$T^2 > \frac{(N_1+N_2-2)t}{N_1+N_2-t-1} F_{\alpha;\ t,\ N_1+N_2-t-1}$, the null hypothesis is rejected and the simultaneous confidence intervals about the vector of mean differences is

$$a'(\bar{X}_1-\bar{X}_2) - \sqrt{a'Sa\ \frac{N_1+N_2}{N_1N_2}\left[\frac{(N_1+N_2-2)t}{N_1+N_2-t-1} F_{\alpha;\ t,\ N_1+N_2-t-1}\right]} \leq a\delta \leq$$

$$a'(\bar{X}_1-\bar{X}_2) + \sqrt{a'Sa\ \frac{N_1+N_2}{N_1N_2}\left[\frac{(N_1+N_2-2)t}{N_1+N_2-t-1} F_{\alpha;\ t,\ N_1+N_2-t-1}\right]}$$

where

$$\frac{(N_1+N_2-2)t}{N_1+N_2-t-1} F_{\alpha;\ t,\ N_1+N_2-t-1} = T^2_{\alpha;\ t,\ N_1+N_2-t-1}.$$

A correlation matrix may be calculated as in the one sample case, except now it is calculated from the pooled variance-covariance matrix. By pooling the two sums of squares and cross products matrices we have assumed that we have homogeneity of variances and thus need not calculate a correlation matrix for each covariance matrix. This assumption will be dealt with shortly.

If the null hypothesis is rejected, a profile analysis may be performed on the two response mean vectors, by asking if the population mean profiles are similar, in the sense that adjacent curve segments are parallel. In behavioral development, for example, parallel curves would indicate similar behaviors, even if at different levels. The null hypothesis is Ho: $C\mu_1 = C\mu_2$.

The test statistic is

$$T^2 = \frac{N_1 N_2}{N_1 + N_2} (\underset{\sim}{\bar{X}}_1 - \underset{\sim}{\bar{X}}_2)' \underset{\sim}{C}' (\underset{\sim}{C}\underset{\sim}{S}\underset{\sim}{C}')^{-1} \underset{\sim}{C} (\underset{\sim}{\bar{X}}_1 - \underset{\sim}{\bar{X}}_2)$$

where

$\underset{\sim}{C}$ = a (K-1) x k patterned matrix, having the effect of subtracting each row in the matrix from the preceding row.

If $T^2 \leq \frac{N_1+N_2-t}{(N_1+N_2-2)(t-1)} F_{\alpha;\ t-1,\ N_1+N_2-t}$ the null hypothesis is not rejected.

An analysis specifically for repeated measures has been worked out for Hotelling's procedure (Morrison 1969, p. 133) in which serial correlation is handled internally by the test statistic. However, this procedure has not been worked out for the case of two samples, has no profile analysis and is limited in scope as to the questions it will answer.

The maximum number of experimental groups that can be analyzed by Hotelling's two-sample test is two. This means that multiple comparisons must be performed for more than two samples, and presents the problem of determining an appropriate α-level for testing the null hypothesis. This is up to the experimenter, but must be decided a priori. If in 100 comparisons, for example, 20 tests turned out to be significant at the $\alpha = .05$ level, then 5 of those tests would be expected to be significant on the basis of chance alone. The α-level should therefore be set at most at the $\alpha = 0.01$ when such comparisons are attempted.

There are several multivariate tests in the literature which allow many treatment groups to be simultaneously compared. There are, however, two problems with such tests. Firstly, they are much less

powerful than Hotelling's T^2 test even when the α-level is adjusted. Secondly, if the results of such a test indicate significant differences, the experimenter has no way of knowing which treatment groups caused the difference. In such a case the only recourse would be pair-wise comparisons to pull apart the differences.

Example 3.2.--In the experimental design of Example 3.1 suppose that we now have two experimental groups each of which is presented with a different degree of biological conditioning and we are interested in asking if larvae respond identically to the different degrees of conditioning.

The raw data matrices are

$$\underset{\sim}{A}_1 = \begin{array}{c} \\ 1 \\ \\ \\ \\ \\ \\ \\ \\ 10 \end{array} \begin{array}{c} \begin{array}{ccc} t_1 & t_2 & t_3 \end{array} \\ \begin{bmatrix} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 0 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 0 \\ 1 & 1 & 1 \\ 0 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \end{bmatrix} \end{array} \qquad \underset{\sim}{A}_2 = \begin{array}{c} \\ 1 \\ \\ \\ \\ \\ \\ \\ \\ 10 \end{array} \begin{array}{c} \begin{array}{ccc} t_1 & t_2 & t_3 \end{array} \\ \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ 1 & 1 & 1 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 0 \\ 1 & 0 & 0 \end{bmatrix} \end{array}$$

$\bar{\underset{\sim}{X}}_1 = [0.9 \quad 0.9 \quad 0.9]$. $\qquad \bar{\underset{\sim}{X}}_2 = [0.5 \quad 0.4 \quad 0.3]$.

The pooled covariance matrix is

$$\begin{bmatrix} .189 & .106 & .078 \\ .106 & .183 & .094 \\ .078 & .094 & .187 \end{bmatrix}$$

The quantity $(\bar{X}_1 - \bar{X}_2) = [0.4 \quad 0.5 \quad 0.6]$.

The test statistic is

$$T^2 = \frac{(10)(10)}{10 + 10} [0.4 \quad 0.5 \quad 0.6] \begin{bmatrix} 8.1347 & -3.8829 & -1.6138 \\ -3.8829 & 9.5404 & -3.5564 \\ -1.6138 & -3.5564 & 8.7436 \end{bmatrix} \begin{bmatrix} 0.4 \\ 0.5 \\ 0.6 \end{bmatrix}$$

$= 11.8652$, and

$$F = \frac{10 + 10 - 3 - 1}{(10 + 10 - 2)3} (11.5156) = 3.5156, \text{ d.f.} = 3, 16.$$

The tabulated critical value of $F_{0.05;\ 3,\ 16} = 3.24$. Since our calculated F is larger than the critical value we reject the null hypothesis and conclude that larvae respond differently to different degrees of conditioning.

The simultaneous confidence intervals are calculated as they were in Example 3.1, except the two sample formula is used. For example if we wanted to look at the first time period, then $\underset{\sim}{a} = [1 \quad 0 \quad 0]$ and the confidence interval becomes 0.7212.

Knowing that the response means are different we now want to test for parallelism between the two curves. This is done by first constricting the (k-1)(k) patterned matrix, $\underset{\sim}{C}$, as follows

$$\underset{\sim}{C} = \begin{bmatrix} 1 & -1 & 0 \\ 0 & 1 & -1 \end{bmatrix}$$

The test statistic is

$$T^2 = \frac{(10)(10)}{10 + 10} [0.4 \quad 0.5 \quad 0.6] \begin{bmatrix} 1 & 0 \\ -1 & 1 \\ 0 & -1 \end{bmatrix} \begin{bmatrix} 1 & -1 & 0 \\ 0 & 1 & -1 \end{bmatrix} \begin{bmatrix} .189 & .106 & .078 \\ .106 & .183 & .094 \\ .078 & .094 & .167 \end{bmatrix}$$

$$\begin{bmatrix} 1 & 0 \\ -1 & 1 \\ 0 & -1 \end{bmatrix}^{-1} \begin{bmatrix} 1 & -1 & 0 \\ 0 & 1 & -1 \end{bmatrix} \begin{bmatrix} 0.4 \\ 0.5 \\ 0.6 \end{bmatrix}$$

$= 1.0$, and

$$F = \frac{10 + 10 - 3}{(10 + 10 - 2)(3 - 1)} (1.0)$$

$= 0.4722$, d.f. = 2, 17.

The tabulated critical value for $F_{0.05;\ 2,\ 17} = 3.59$. Since the calculated F is less than the tabulated value the null hypothesis cannot be rejected and we conclude that the curves are parallel. This indicates that although the response mean vectors are significantly different, these organisms are behaving the same way but at different levels. That is, there is no interaction between response and age of larvae.

The Assumptions Underlying Hotelling's Procedure

The assumptions underlying Hotelling's T^2 Statistic are: (1) multivariate normal distribution, (2) equality of

variance-covariance matrices, and (3) serially uncorrelated data. These assumptions must be satisfied.

Multivariate Normal Distribution

Just as in the univariate case, data for multivariate analysis must be normally distributed. Procedures for testing this assumption are handled by Sokal and Rohlf (1969) and appropriate transformations are discussed, which are the best ways to correct for lack of normality. If non-normality is present, then the probability value for F is an underestimate which causes errors in the direction of announcing too many significant results (Cochran 1947). In addition to its effects on the validity of tests of significance, non-normality is accompanied by a loss in efficiency in the estimation of treatment effects and corresponding loss of power in the F- or t-tests. Sokal and Rohlf (1969), however, show that the consequences of non-normality are not too serious. Only very skewed distributions would have a marked effect on the significance level of the F-test or on the efficiency of the design.

Given certain prerequisites, data that is not normally distributed may be used with Hotelling's T^2 Statistic. For example, the data discussed in Examples 3.1 and 3.2 is binomial data, which is a discrete distribution not a continuous one. It would seem, therefore, that this data should be transformed to fit a normal distribution. However, the crucial part of Hotelling's test is the difference vector $(\underset{\sim}{\bar{X}}-\underset{\sim}{\bar{X}}_o)$ in the one sample or $(\underset{\sim}{\bar{X}}_1-\underset{\sim}{\bar{X}}_2)$ in the two sample. Although the raw data is discrete, i.e., ones and zeros, the data being tested comes from $(\underset{\sim}{\bar{X}}-\underset{\sim}{\bar{X}}_o)$ or $(\underset{\sim}{\bar{X}}_1-\underset{\sim}{\bar{X}}_2)$ which may yield any number

between 1 and 0. This then becomes a continuous distribution, as N approaches ∞.

The Central Limit Theorem states that as sample size increases, the means of samples drawn from a population of any distribution will approach the normal distribution. There are not very many statisticians, however, willing to categorically state how big the sample size must be before this theorem is true, but a general rule of thumb is > 30. However, for illustrative purposes smaller samples are described in Examples 3.1 and 3.2. For actual test data calculate, a priori, a sample size necessary to satisfy the assumption of normality based upon the Central Limit Theorem. The procedure (personal communication with Dr. John Gill of Michigan State University) is

$$N = 3/\hat{W}(1-\hat{W})$$

where N = sample size

$\hat{W}$ = any value the experimenter would like to be able to differentiate. This might be done by picking the smallest difference one wishes to be able to detect.

Let us suppose we have run a pilot experiment in the two choice situations described earlier to determine Galleria's preference to the highest degree of conditioning we intend to test, and find that the largest response mean in the mean vector is 0.96. This is $\hat{W}$. It is now possible to calculate the N that would be necessary, given that $\hat{W} = 0.96$, to satisfy the assumption of normality. The result is

$$N = 3/0.96(1-0.96)$$

$$= 78.125.$$

Therefore, an N greater than 78 would be required.

Equality of Variance-Covariance Matrices

A test for heterogeneous variance is not necessary in the one sample test. Hotelling (1931) originally devised the statistic without the underlying assumption that the variances within a group are homogeneous. This is reasonable since the test is designed to examine multivariate factors among which homogeneous variances would not be expected.

When comparing two experimental groups, however, the assumption of equal variance-covariance matrices must be satisfied. Just as in the univariant case where the assumption is made that the observations are normally distributed about their population mean values with constant variance, so an analogous assumption that the variates are multinormally distributed about their mean values with constant variance-covariance matrices is made in the multivariate analysis of variance. Cochran (1947) has shown that if ordinary analysis of variance methods are used when the true error variance differs from one observation to another there will be a loss of efficiency in the estimates of treatment effects as well as a loss of sensitivity in tests of significance. The validity of the F-test for all treatments is probably least affected, but t-tests from a pooled error may seriously distort the significance levels.

Box (1950) has devised a method for testing the assumption of homogeneous variances in multivariate analysis.

Example 3.3.--Let us return to Example 3.2 and calculate Box's test for homogeneous variances.

$$M = N \log_e |\underset{\sim}{S}| - \sum_k (V_k \log_e |\underset{\sim}{S}_k|),$$

where

$N = V_k$, the total of the degrees of freedom,

$\underset{\sim}{S}_k$ = the unbiased estimate of each covariance matrix being compared,

V_k = degrees of freedom,

$\underset{\sim}{S}$ = the pooled variance or covariance matrix, and

$||$ = the symbol for determinants.

In Example 3.2,

$$\log_e|\underset{\sim}{S}_1| = -6.948348, \quad V_1 = 9$$

$$\log_e|\underset{\sim}{S}_2| = -6.1862086, \quad V_2 = 9$$

$$\log_e|\underset{\sim}{S}| = -5.9251272, \quad N = 18$$

$$M = 18(-6.1862086) - 9(-6.948348) - 9(-6.1862086)$$

$$M = 6.8592.$$

Calculate

$$A_1 = \frac{2t^2 - 3t - 1}{6(t+1)(k-1)} \left(\sum_k 1/V_1 - 1/N\right),$$

where k = the number of matrices being compared.

$$F_1 = 1/2\ (k-1)t(t+1).$$

Therefore,

$$A_1 = \frac{2(3)^2 + 3(3) - 1}{6(3+1)(1-1)}$$

$$= 0.1806, \text{ and}$$

$$F_1 = 1/2\ (1-1)3(3+1)$$

$$= 6,$$

and $(1-A_1)M$ is distributed as X^2 with f_1 degrees of freedom, or

$$(1-0.1806)6.8592 = 5.6204$$

In this case $A_1 = 0.1806$, $f_1 = 6$, $(1-A_1)M = 5.6204$ is referred to tables of X^2 with 6 degrees of freedom. The probability for the occurrence of a value as great or greater than this, when the variances and covariances are in fact homogeneous from one group to

the next, is thus between 0.5 and 0.1, and there is therefore no reason to doubt the homogeneity of the data.

The chi-squared approximation is good if k and/or t do not exceed 4 or 5 or if some of the degrees of freedom are small. If, however, k and/or t are greater than 5 or the degrees of freedom are large, Box proposes an F-distribution approximation where

$$A_2 = \left[\frac{(t-1)(t+2)}{6(k-1)} \sum_k 1/V_k^2\right] - 1/N^2$$

and M/b is referred to an F-table with f_1 and f_2 degrees of freedom where

$$f_2 = \frac{f_1 + 2}{A_2 - A_1^2} \quad \text{and} \quad b = \frac{f_1}{1 - A_1 - f_1/f_2}$$

It will be noticed that Box's approximation allows the simultaneous analysis of any number of matrices, but does not provide a method for distinguishing which matrix is causing problems when the test is heterogeneous. Therefore, it is necessary to perform pairwise comparisons in this event.

If the test proves to be heterogeneous there is no approximate method available for Hotelling's two-sample test and the test should be run as if the variances were homogeneous. If this is done a decision must be made pertaining to the validity of the significance test. If two covariance matrices are heterogeneous the pooled covariance matrix will be smaller than one and larger than the other. There will thus be a loss in sensitivity because the variances have been inflated, making it more difficult to reject the null hypothesis. Therefore, we can be confident of a significance test in which the null hypothesis has been rejected, at the usual α-level of 0.05.

If <u>a priori</u> an experimenter suspects finding heterogeneity of variance he should increase the sample size. Ito and Schull (1964) have demonstrated that, for Hotelling's two-sample statistic, as N

approaches infinity the test statistic and significance tests are unaffected by heterogeneous variances. The general rule of thumb is that N must be greater than or equal to 30 for this to hold true.

Serially Uncorrelated Data

The greatest difficulty with longitudinal data is determining if the error terms are correlated and what to do if they are. However, it does not necessarily follow that longitudinal data is correlated. If the assumption of independence is satisfied, the analytical procedure is valid whether or not the observations themselves are serially correlated. Cochran (1947) has shown that if correlation exists the following are the consequences: (1) if the correlation is positive, the treatment means are less accurate than the mean of an independent series, but are estimated to be more accurate, and (2) if correlation is negative, these conditions are reversed.

Durbin and Watson (1950, 1951) and Watson (1955) have worked out several regression models for testing the assumption of serially uncorrelated data. There are several of these tests, depending on the kind of data or experimental design, and the reader is directed to the original papers. However, for the purposes of my design and resultant discrete data the Durbin-Watson "One-and-Two-Way Classification was used (see pp. 166-168 of Durbin-Watson 1951). The calculations are much too laborious to be presented at this time, but entail a regression model consisting of

$$Y_{ij} = \mu + \alpha_i + \beta_j + \Sigma_{ij}$$

where

Y_{ij} = the observation in the i^{th} column and j^{th} row,

$\mu_{1\alpha ij} + \beta$ = constants, and

Σ_{ij} = the error term.

Least squares estimates of μ, α_i and β_i are calculated and designated as m, A_i-m and b_j-m

where

m = the sample mean of all observations,

A_i = the mean observation in the i^{th} column,

b_j = the mean observation in the j^{th} row.

Thus the residuals are given by

$$Z_{ij} = Y_{ij} - A_i - b_j + m, \text{ and}$$

the test is made by calculating

$$d = \frac{\Sigma(\Delta Z_{ij})^2}{\Sigma Z^2_{ij}},$$

where ΔZ_{ij} = the first differences of the residuals when arranged as a single time series.

The calculated d-value is compared to a lower (d_L) and upper (d_U) range of critical values. If $d < d_L$ there is positive correlation; if $d > d_U$ no correlation exists. If $d_L \leq d \leq d_U$ the test is inconclusive. Durbin and Watson (1951) have calculated several tables of upper and lower d-values. However, most of my research deals with large matrices and I had to generate other tables in situations where k' (the number of time units) was greater than 5. This was done on the computer using Durbin and Watson's (1950) formulas on p. 427.

Regardless of the outcome of the Durbin-Watson test for serial correlation, Hotelling's T^2 statistic is run normally. If correlation exists, then by examining the variance-covariance matrices we can make a decision about the significance tests' validity. Positive serial

correlation means that the variances are over-estimates making it difficult to reject the null hypothesis, whereas negative correlation means the variances have been under-estimated making it easy to reject the null hypothesis. Therefore, with positive correlation the significance tests should be run at the $\alpha = 0.05$ level and at the $\alpha = 0.01$ level when negative correlation is the problem.

However, tests for negative correlation are rarely run unless the experimenter has an a priori reason to suspect it. For example, it is a common practice in econometric work (Durbin and Watson 1951) to analyze the first differences of the observations rather than the observations themselves, on the ground that the serial correlation of the transformed errors is likely to be less than that of the original errors. It is possible that the transformation has over-corrected, thus introducing negative serial correlation into the transformed errors.

Summary

Statistical procedures, and the assumptions underlying them, are presented for the multivariate analysis of longitudinal data. Hotelling's One and Two Sample T^2 Statistics for Multivariate Analysis are outlined, illustrating a procedure for treating time as a multi-dimensional variate. Emphasized are procedures for testing the assumptions of homogeneous variances and non-serially correlated data. The major objective is to arm behavioral biologists with the necessary analytical techniques enabling logical decisions about tests of significance on repeated individual measurements over time.

CHAPTER IV

BIOLOGICAL CONDITIONING AND SPATIAL DISTRIBUTION IN GALLERIA MELLONELLA (L.) LARVAE

Introduction

Stimuli affecting spatial distribution are such environmental parameters as light, temperature and humidity (Allee 1926; Heatwole 1962; Surtees 1963a, 1964e & f; Friedlander 1964; Warburg 1964), food (Park 1938; Brown 1946; Loschiavo 1952; Wynne-Edwards 1962; Beck 1960), sex and genetic factors (Wellington 1957; Naylor 1959; McDonald and Fitting 1965; Ogden 1969), and density (Long 1953; Wellington 1957; Naylor 1959; Surtees 1963a, b & c, 1964a, b, c, e & f). This paper, however, is concerned with the relationship between biological conditioning and spatial distribution.

Biological conditioning refers to changes produced in a medium by organisms living therein. The results of such homotypic conditioning are physiological or behavioral. The physiological effects of biological conditioning have been extensively studied (Uvarov 1938; Allee 1931, 1934; Adolph 1931; Park 1932, 1934, 1935, 1936a & b; Brown 1946, 1951; Long 1953). It has been demonstrated that biological conditioning affords protection from toxic substances, is involved in control of sex, affects morphological changes and growth, and affects population dynamics, such as natality and

mortality. Historically, biological conditioning has been considered to be a population phenomenon. It is an expression of population density and is a normal and inevitable result of population growth. As populations grow and maintain themselves, they of necessity modify their environment and the environment in turn modifies them. However, individuals also condition their environment and it has not yet been demonstrated if the effects of population conditioning are different from the effects of individual conditioning. Since biological conditioning is a result of the organisms themselves and since the degree of conditioning is a function of the number of organisms present or of the length of time an organism is present, biological conditioning and animal-animal interactions have been considered as an inescapable unit. However, they can be separated by experimental procedures. The effects of either, or the interaction between the two, may alter the behavior of individuals with resulting effects on populations.

Biological conditioning has been demonstrated to affect spatial distribution of populations of organisms, particularly invertebrates (see Chapter I). It has been shown in Tribolium (Park and Woolcott 1937; Park 1948) that homotypic conditioning alters the movements of these beetles and that adult populations of Tribolium seem to prefer weakly-conditioned medium. Naylor (1959 and 1965) extended these findings to include animal-animal encounters, such as interactions between the sexes, and homotypic conditioning, and he found that the distribution of Tribolium confusum and T. castaneum was only explainable when all three factors were accounted for. The interaction between these three variables seems a common phenomenon among

invertebrates, particularly insects. For example, Long (1953, 1955) with butterfly larvae and Wellington (1957) with larvae of the tent caterpillar both demonstrated similar interactions.

Although biological conditioning affects spatial distribution and distribution may also be altered by other variables, the interactions between animal-animal interactions and homotypic conditioning remain confounded. Studies dealing with biological conditioning deal with whole populations, and little, if any, data have been collected on the effects on individual behavior.

It is the purpose of this paper to look at the role of homotypic conditioning in the dispersionary behavior of isolated Galleria mellonella (L.) larvae, the assumption being that before we can understand population behavior we must first understand individual behavior. The general hypothesis being tested is that response of isolate larvae to biological conditioning will change as a function of the degree of conditioning and age of the test larva. In testing this hypothesis a bioassay of behavioral preferences to conditioning was developed.

Materials and Methods

Husbandry

Rearing Galleria mellonella is a relatively simple task and was described in Chapter II. All experimental larvae were drawn from the colony dishes which were maintained in darkness in incubators at 31±1°C and 75% R.H. Due to the manner in which egg hatches were collected (see Chapter II), the age of larvae within any colony dish had a maximum possible variability of 24 hours. It was therefore

necessary to have a criteria for consistently determining age from hatch to hatch. Weight of larvae was used as the criteria. Table 4.1 shows the mean weights of 500 larvae, at each age, randomly selected from appropriate colony dishes as well as the corresponding weights of larvae actually used for experimental purposes. Figure 4.1 shows the size distribution of larvae corresponding to Table 4.1.

Apparatus

The experimental apparatus consisted of 100 x 25 mm plastic petri dishes with two 4±0.05 gram food lumps in each dish. Food was prepared as described in Chapter II. Within any one dish the food lumps were placed on opposite sides of the dish so that test larvae could be introduced between them and allowed to make a choice.

Experimental Procedures

The experimental design is shown in Figure 4.2. Four conditioning periods (1, 3, 5, and 7 days) and three ages of conditioning larvae (6, 9, and 12 days of age) were used. Preference tests were run with 7, 11, and 13 day old test larvae for each conditioning age and/or period. Each box in the design has an identification code (1E, 2E, 3E, and 37C) where E stands for experimental group and C for control group. There is one control for every three experimental groups based on days of conditioning. For example, 37C is the control for groups 1E, 2E, and 3E at one day of conditioning. Each group has a sample size of 80.

The date in each box of Figure 4.2 indicates the day on which the test was begun for each group. Due to the size of the

Table 4.1
Larval weights corresponding to the larvae in Figure 4.1. Weights were calculated for 500 randomly selected larvae of a particular age as well as for the larvae of a particular age actually used for experimentation.

Age (Days)	Randomly Selected [Mean weights(mg) ± standard deviation]	Experimental Larvae [Mean weights(mg) ± standard deviation]
6	0.53 ± 0.012	0.71 ± 0.009
7	1.27 ± 0.029	1.49 ± 0.010
9	5.29 ± 0.090	6.33 ± 0.069
11	12.45 ± 0.221	12.72 ± 0.014
12	22.14 ± 0.710	22.56 ± 0.320
13	34.40 ± 0.760	33.82 ± 0.221
15	57.60 ± 0.700	55.40 ± 0.250

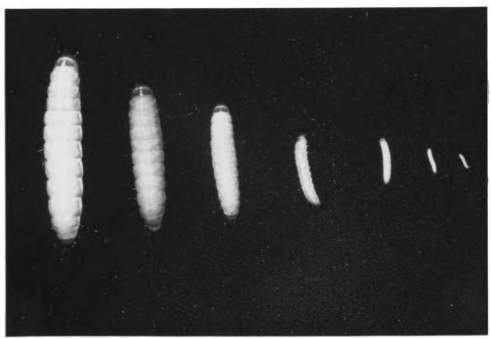


Figure 4.1
From right to left, these are 6, 7, 9, 11, 12, 13, & 15 day old larvae used for conditioning and testing preference for conditioning. Note the size distribution of the larvae.

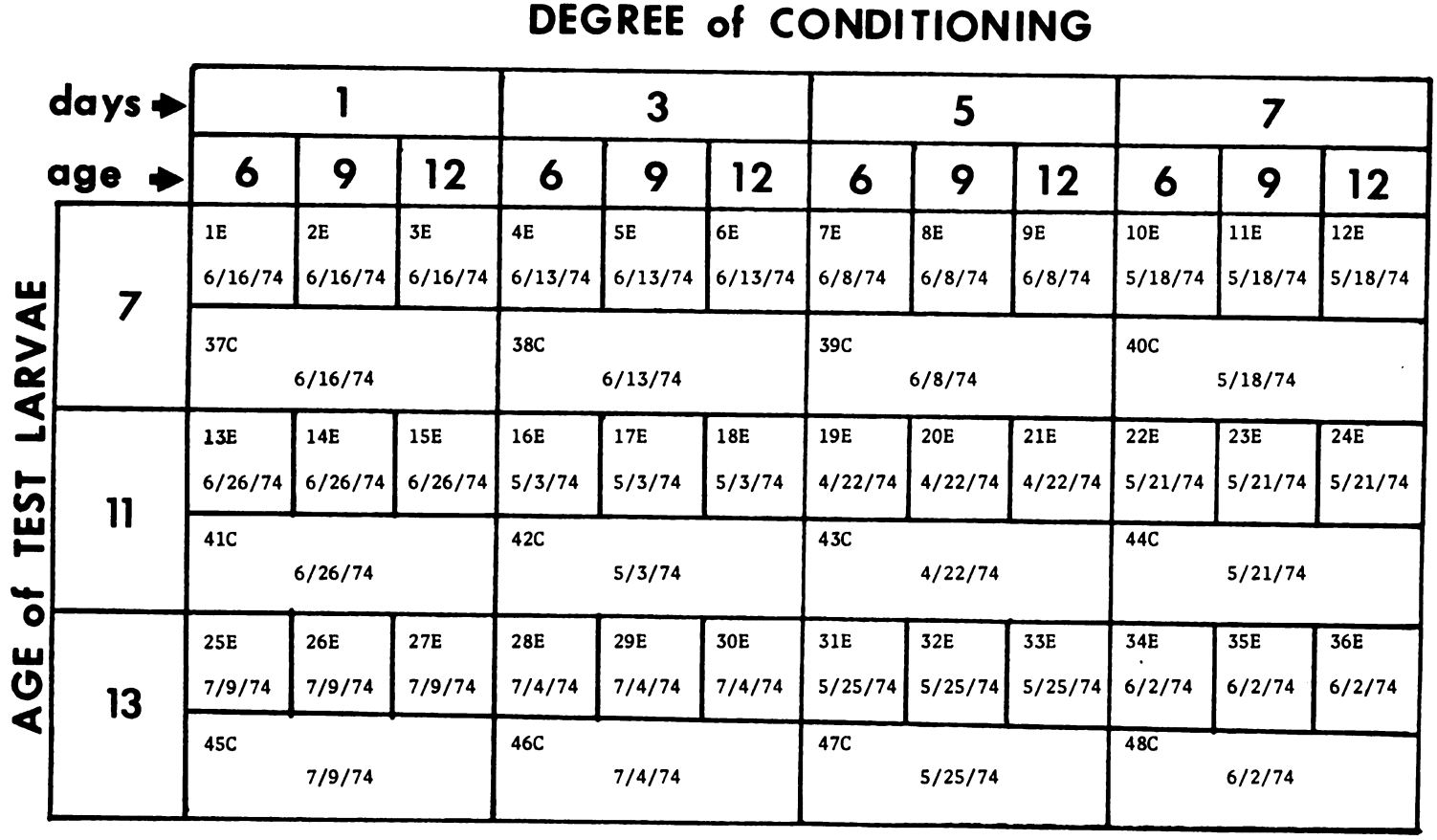


Figure 4.2

Experimental design, showing the identification labels and the date when each experimental (E) group and Control (C) group was run.

experimental design and the total number of larvae required (total N = 3840) it was necessary to test groups at different times and with different populations (hatches) of larvae. Therefore, the test date for every block of three experimental groups and their control was randomly determined, a priori. Every four groups form a natural entity of days of conditioning, age of conditioning larvae, and age of test larvae and were therefore run at the same time. For example, 1E, 2E, 3E, and 37C or 19E, 20E, 21E, and 43C were each run as a unit.

Experimental group 8E has been chosen to elucidate the experimental procedures. Food was prepared as discussed in Chapter II, Table 2.1, and 160, 4±0.05 gram food lumps were weighed on a Mettler P120 Scale. These food lumps were flattened on wax paper until they were approximately 6 mm thick and 157 mm in circumference. One food lump was then placed in each of 160, 150 x 25 mm plastic petri dishes, the lump touching the periphery of the dish. In 80 of these dishes a conditioning larva was added, in this case a 9-day old larva. The other dishes have no conditioning larva. All dishes were then put in a Jamesway Single Stage Incubator-Hatcher, Model 252B, for the length of time conditioning was to occur; in this case 5 days.

At the end of the 5 day period the following manipulations were performed. The conditioning larva, which is now 14 days old, is removed from each of the 80 dishes by gently tapping the silk tunnels it has spun with a pair of forceps. This causes the larva to poke its head out and it can be carefully removed with forceps. Once all larvae are removed, the 80 non-conditioned food lumps are placed one in each of the conditioned dishes opposite the conditioned lump. This

is done wearing surgical gloves since pilot experiments indicated larvae will selectively respond to food handled with bare hands. The distance between food lumps is approximately 35 mm across the center of the dish. A small black dot is placed on the side of the petri dish opposite one of the food lumps for identification purposes. The food lumps receiving the identification dot were randomly selected so as to alleviate the possibility of response to the dots. An identification number was also put on the top of each dish.

Once the two food lumps are in the same dish, a single, in this case 7 day old, larva of proper size is removed from the appropriate colony dish and placed in the petri dish between the lumps and the top is placed on each dish. The total procedure of removing the conditioning larvae, adding the plain food lumps, and seeding all 80 dishes with a test larva takes 5 minutes. At the end of the 5 minute period the location of each larva is recorded and the dishes placed in a Napco (Model 330) incubator at 32±1°C and 75% R.H. in darkness. The incubator holds 8 shelves of 80 dishes per shelf with the dishes stacked 5 deep.

Every 12 hours the dishes were removed from the incubator and data collected as to which food lump the larvae were residing in. The dependent variable, therefore, is position of the larvae over time. Except during the wandering phase, larvae were never found between food lumps. Every 24 hours the position of the dishes on each shelf as well as shelf position in the incubator was randomized to prevent any larval responses to discontinuities of temperature, humidity, or light within the incubator.

In order to record position of the larvae it was necessary to bring the dishes into the light for 5 minutes, every 12 hours. There is some indication that Galleria larvae are photonegative and I may have been recording the preferred food lump in which to escape from light. However, differences in response to degree of conditioning are still evident. Data were collected every 12 hours until pupation occurred, at which time the experiment was terminated.

The control group for 8E, namely 39C, was set up exactly as the experimental group except that none of the food lumps were conditioned. The controls, therefore, control for food differences and the handling of one of the food lumps at the end of the conditioning period. The response of a larva in a control group will be random if these variables have no effect on distribution or preferences.

Every group in Figure 4.2 was set up exactly as just described, the only differences being the number of days of conditioning, age of conditioning larvae, and age of test larvae.

For purposes of ease in referring to this experimental design and manipulations, I will refer either to the group number, 1E or 10E, or I will use a CAT-value, where C = the number of conditioning days, A = age of conditioning larva, and T = age of test larva. Therefore, CAT = 1, 6, 7 refers to an experiment in which 1 day of conditioning was done by a 6 day old larva and the response of a 7 day old larva was tested. For group 8E, CAT = 5, 9, 7; for group 27E, CAT = 1, 12, 13 and so on. If multiple comparisons are desired, the CAT-value can be used to indicate these as well. For example, if we wanted to look at 1, 3, 5, and 7 days of conditioning done by a 6 day old larva

at the start of conditioning and tested with a 7 day old larva then CAT = (1, 3, 5, 7), 6, 7.

In all Tables and Figures in this paper test larvae are referred to as 7, 11, or 13 days old. It must be remembered, however, that these are the ages when a particular experiment is started, and that, for example, four days into an experiment a 7 day old larva is 11 days old, an 11 day old larva is 15 days old, and a 13 day old larva is 17 days old. The abscissars in all graphs in this paper are calibrated in days, following initiation of the test not age of test larva.

Analysis

The dependent variable chosen for analysis within each experimental group is the mean response to conditioned food over time. Each experimental group has 80 dishes with 1 larva per dish. If a larva is in the conditioned lump it received a score of 1, if in the non-conditioned (plain) lump it received a 0. In the case of 8E, for example, this would generate an 80 x 20 matrix whose entries are either one's or zero's, 80 being the number of dishes or larvae and 20 being the number of 12 hour intervals. A mean for each 12 hour interval can be calculated which generates a 1 x 20 vector whose entries are the mean number of larvae in conditioned food at each 12 hour time interval. For actual analysis, however, 24 hour intervals were utilized, since there was no difference in response from AM (morning) to PM (afternoon) readings (Table 4.2). The wandering phase was also not included in the analysis because wandering larvae exhibited atypical behavior. This was done because some data

Table 4.2

Twelve experimental groups in Figure 4.2 were randomly selected and their AM and PM mean vectors were compared using Hotelling's One(') and Two(*) Sample analyses.

Group		Time in Days 1	2	3	4	5	6	7	8	9	10	F1	F2	D	Box's Test
	$\bar{X}_{AM}$	.487	.575	.575	.625	.662	.637	.625	.662	.612	.625		0.42	1.40*	
2E	$\bar{X}_{PM}$	.487	.587	.612	.650	.662	.650	.625	.625	.637	.587			1.50*	
	S^2_{Pooled}	.253	.246	.244	.234	.226	.232	.237	.232	.237	.241				
	$\bar{X}_{AM}$	.662	.725	.762	.812	.900	.875	.900	.925	.912	.750		0.26	1.34*	
6E	$\bar{X}_{PM}$	.662	.725	.787	.812	.887	.875	.912	.900	.875	.700			1.48*	
	S^2_{Pooled}	.226	.202	.176	.154	.096	.111	.086	.081	.096	.201				
	$\bar{X}_{AM}$	.637	.750	.762	.812	.750	.787	.712	.775	.775	.750		0.90	1.36*	
7E	$\bar{X}_{PM}$	.625	.750	.787	.750	.812	.787	.762	.775	.775	.712			1.37*	
	S^2_{Pooled}	.236	.190	.176	.172	.172	.169	.195	.177	.177	.199				
	$\bar{X}_{AM}$	.725	.875	.837	.900	.850	.900	.912	.887	.925	.812	1.74	1.19	1.55*	
10E	$\bar{X}_{PM}$	.725	.800	.887	.837	.875	.912	.900	.875	.925	.737			1.65*	
	S^2_{AM}	.202	.111	.138	.091	.129	.091	.081	.101	.070	.154				H
	S^2_{PM}	.202	.162	.101	.138	.111	.081	.091	.111	.070	.196				
	S^2_{Pooled}	.202	.136	.119	.114	.120	.085	.086	.106	.070	.175				

'$F1_{(10,70)} = 2.24$ at $P = .025$

*$F2_{(10,149)} = 2.15$ at $P = .025$; $D_L = 1.71$ and $D_U = 2.28$ at $P = .05$. (I = inconclusive).

The expected vector for the One Sample(') test came from the group with the largest covariance vector.

HHeterogeneous variances ($P \leq .05$).

Table 4.2 (cont'd.)

Group		Time in Days 1	2	3	4	5	6	F2	D	Box's Test
13E	$\bar{X}_{AM}$	.437	.525	.475	.512	.513	.550	0.45	2.00I	
	$\bar{X}_{PM}$	.437	.512	.487	.512	.512	.512		1.97I	
	S^2_{Pooled}	.249	.253	.253	.253	.252	.252			
16E	$\bar{X}_{AM}$	.725	.700	.700	.700	.700	.737	0.46	2.01I	
	$\bar{X}_{PM}$	.725	.712	.687	.675	.687	.687		1.89I	
	S^2_{Pooled}	.202	.210	.215	.217	.215	.207			
20E	$\bar{X}_{AM}$	.637	.800	.825	.887	.912	.912	0.14	1.91I	
	$\bar{X}_{PM}$	.637	.812	.850	.887	.887	.900		1.90I	
	S^2_{Pooled}	.234	.158	.138	.101	.091	.085			
23E	$\bar{X}_{AM}$	.754	.900	.925	.912	.912	.912	0.12	2.07I	
	$\bar{X}_{PM}$	.762	.912	.925	.912	.925	.900		1.90I	
	S^2_{Pooled}	.183	.086	.070	.081	.076	.086			

*$F2_{(6,153)} = 2.51$ at $P = .025$; $D_L = 1.83$ and $D_U = 2.16$ at $P = .05$ (I=inconclusive).

HHeterogeneous variance ($P \leq .05$).

Table 4.2 (cont'd.)

Group		Time in Days 1	2	3	4	F1	F2	D	Box's Test
	$\bar{X}_{AM}$	.642	.662	.662	.662		0.03	2.06I	
26E	$\bar{X}_{PM}$	.662	.662	.650	.650			2.21	
	S^2_{Pooled}	.226	.226	.228	.228				
	$\bar{X}_{AM}$	.725	.800	.875	.887		1.12	2.23	
29E	$\bar{X}_{PM}$	.750	.825	.875	.837			2.37	
	S^2_{Pooled}	.190	.154	.111	.119				
	$\bar{X}_{AM}$	.850	.925	.937	.950	1.35	1.07	2.00I	
	$\bar{X}_{PM}$	.850	.950	.937	.925			2.20	
33E	S^2_{AM}	.129	.070	.059	.048				H
	S^2_{PM}	.129	.048	.059	.070				
	S^2_{Pooled}	.129	.059	.059	.059				
	$\bar{X}_{AM}$	.650	.775	.737	.737		0.16	2.25	
36E	$\bar{X}_{PM}$	.650	.762	.750	.737			2.34	
	S^2_{Pooled}	.230	.180	.193	.196				

'$F1_{(4,76)} = 2.96$ at P = .025. I D-value was inconclusive.

*$F2_{(4,155)} = 2.88$ at P = .025; $D_L = 1.89$ and $D_U = 2.10$ at P = .05.

The expected vector for the One Sample(') test came from the group with the largest covariance vector.

H heterogeneous variances ($P \leq .05$).

reduction was necessary, and will be discussed shortly. Therefore, the data matrices are 80 x 10 for 7 day old test larvae, 80 x 6 for 11 day old test larvae, and 80 x 4 for 13 day old test larvae. The respective mean response vectors are 1 x 10, 1 x 6, and 1 x 4.

Statistical analysis is by means of Hotelling's One- and Two-Sample T^2 Statistics for Multivariate Analysis (see Chapter III), although some Chi-Square and ANOVA analyses are employed. An a priori decision about the α-level for tests of significance had to be made. This was necessary because multiple comparisons were to be made, positive serial correlation was suspected and the variances between groups may be heterogeneous. Serial correlation and heterogeneous variances make it more difficult to reject the null hypothesis, whereas multiple comparisons increase the probability of rejecting a null hypothesis on chance alone. However, the maximum number of comparisons made with any one group is 10, which means we would expect less than a 0.5 in 10 chance of a significant difference on the basis of chance alone at the 0.05 level. It was therefore, determined to use an α-level of 0.05. However, the null hypothesis is two-tailed and the α-level is actually 0.025. A two-tailed hypothesis was utilized because there was no a priori basis upon which to assume response to conditioning to be attraction or repulsion.

After making comparisons with Box's test for heterogeneous variances, I found that, almost without exception, the variances in each comparison to be heterogeneous. In Hotelling's Two-Sample Statistic, a covariance matrix is calculated by pooling the two covariance matrices of the groups being compared. If the respective

covariance matrices are homogeneous, the pooled covariance matrix is the best estimate of the two. If the two covariance matrices are heterogeneous, as they are in my data, then the pooled covariance matrix is inflated and is not the best estimate. Ito and Schull (1964), however, indicate that heterogeneity of variances does not affect the power of the test or the level of significance if N is very large, however, large N is not well defined. If the null hypothesis is rejected when the variances are heterogeneous we may be sure of this decision, but if it is not rejected then we may be accepting false null hypotheses.

Because of these considerations, I also ran Hotelling's One-Sample statistic on those comparisons with heterogeneous variances and in which the Two-Sample statistic had not rejected the null hypothesis. In this procedure the mean vector of the group with the greater variance is used as an expected vector (EV.) and is compared to the other test mean vector using the covariance (CV.) of the group with the smaller variance. This procedure has the effect of letting the smaller covariance matrix be the "best estimate" of the two groups being compared and asking whether those groups are still not different. If the groups are still not different then we know that it is not because of heterogeneous variances. If the two groups are found to be different with the One-Sample procedure, then we know that non-rejection of the null hypothesis with the Two-Sample test was a result of heterogeneous variances. This piece of information coupled with examination of the variance-covariance vectors in Table 4.3 gives us additional information about the behavior of the experimental animals

Table 4.3

Results of Hotelling's One Sample comparison of each group from Figure 4.2 with an expected vector whose entries are all 0.5 to determine which groups are random. Mean vectors($\bar{X}$), estimated variance vectors(S^2), F-values, and D-values for serial correlation are given.

		Time in Days											
Group		1	2	3	4	5	6	7	8	9	10	F	D
1E	$\bar{X}$	.500	.550	.437	.450	.462	.475	.462	.475	.487	.475	0.86	1.55*
	S^2	.253	.251	.249	.251	.252	.253	.252	.253	.253	.253		
2E	$\bar{X}$	.478	.587	.612	.650	.662	.650	.625	.625	.637	.587	1.31	1.51*
	S^2	.253	.245	.240	.230	.226	.230	.237	.237	.234	.245		
3E	$\bar{X}$	.512	.625	.650	.675	.700	.687	.737	.712	.737	.675	3.12**	1.54*
	S^2	.253	.237	.230	.222	.213	.218	.196	.207	.196	.154		
37C	$\bar{X}$	.512	.475	.487	.562	.537	.550	.500	.512	.500	.475	0.94	1.34*
	S^2	.253	.253	.253	.249	.252	.251	.253	.253	.253	.253		
4E	$\bar{X}$	.550	.662	.650	.637	.625	.625	.625	.612	.550	.462	1.94	1.42*
	S^2	.251	.226	.230	.234	.237	.237	.237	.240	.251	.252		
5E	$\bar{X}$	.562	.700	.762	.750	.800	.837	.800	.812	.712	.612	8.59**	1.39*
	S^2	.249	.213	.183	.190	.162	.138	.162	.154	.207	.240		
6E	$\bar{X}$	.662	.725	.787	.812	.887	.875	.912	.900	.875	.700	23.97**	1.48*
	S^2	.226	.202	.169	.154	.101	.111	.081	.091	.111	.213		
38C	$\bar{X}$	.525	.487	.487	.462	.500	.475	.500	.575	.475	.387	1.84	1.53*
	S^2	.253	.253	.253	.252	.253	.253	.253	.247	.253	.240		
7E	$\bar{X}$	.625	.750	.787	.750	.812	.787	.762	.775	.775	.712	6.82**	1.37*
	S^2	.237	.190	.169	.190	.154	.169	.183	.177	.177	.112		
8E	$\bar{X}$	.625	.737	.837	.837	.837	.862	.856	.862	.837	.712	11.40**	1.46*
	S^2	.237	.196	.138	.138	.138	.120	.129	.120	.138	.207		
9E	$\bar{X}$	.637	.862	.837	.850	.925	.950	.912	.962	.925	.837	74.72**	1.16I
	S^2	.234	.120	.138	.129	.070	.048	.081	.037	.070	.138		
39C	$\bar{X}$	.462	.512	.462	.537	.462	.487	.475	.487	.437	.350	1.26	1.38*
	S^2	.252	.253	.252	.252	.252	.253	.253	.253	.249	.230		
10E	$\bar{X}$	.725	.800	.887	.837	.875	.912	.900	.875	.925	.737	23.48**	1.65I
	S^2	.202	.162	.101	.138	.111	.081	.091	.111	.070	.196		
11E	$\bar{X}$	.625	.762	.875	.875	.950	.925	.937	.862	.862	.812	48.62**	1.48*
	S^2	.237	.183	.111	.111	.048	.070	.059	.120	.120	.154		
12E	$\bar{X}$	.700	.687	.712	.725	.800	.850	.875	.862	.812	.725	12.76**	1.55*
	S^2	.213	.218	.207	.202	.162	.129	.111	.120	.154	.202		
40C	$\bar{X}$	.562	.525	.537	.500	.525	.500	.500	.525	.437	.337	1.58	1.41*
	S^2	.249	.253	.252	.253	.253	.253	.253	.253	.249	.226		

*$F_{(10,70)} = 2.24$ at P = .025; $D_L = 1.71$ and $D_U = 2.28$ at P = .05.

**$F_{(10,70)} = 2.59$ at P = .01.

I D-value was inconclusive.

Table 4.3 (cont'd.)

Group		Time in Days 1	2	3	4	5	6	F	D
13E	$\bar{X}$	.437	.512	.487	.512	.512	.512	0.49	1.97I
	S^2	.249	.253	.253	.253	.253	.253		
14E	$\bar{X}$	.637	.687	.650	.725	.687	.700	4.35**	1.98I
	S^2	.234	.218	.230	.202	.218	.213		
15E	$\bar{X}$	.575	.687	.687	.725	.775	.762	7.07**	1.72I
	S^2	.247	.218	.218	.202	.177	.183		
41C	$\bar{X}$	.457	.500	.525	.475	.525	.537	0.89	1.97I
	S^2	.253	.253	.253	.253	.253	.252		
16E	$\bar{X}$	.725	.712	.687	.675	.687	.687	5.28**	1.89I
	S^2	.202	.207	.218	.222	.218	.218		
17E	$\bar{X}$	.737	.850	.800	.850	.812	.800	17.70**	1.85I
	S^2	.196	.129	.162	.129	.154	.162		
18E	$\bar{X}$	.712	.875	.912	.950	.900	.950	66.80**	1.76*
	S^2	.207	.111	.081	.048	.091	.048		
42C	$\bar{X}$	.475	.475	.425	.412	.450	.437	0.85	1.89I
	S^2	.253	.253	.247	.245	.251	.249		
19E	$\bar{X}$	.637	.812	.825	.862	.850	.812	14.82**	1.95I
	S^2	.234	.154	.146	.120	.129	.154		
20E	$\bar{X}$	.637	.812	.850	.887	.887	.900	27.32**	1.90I
	S^2	.234	.154	.129	.101	.101	.091		
21E	$\bar{X}$	.675	.937	.925	.912	.875	.850	78.01**	2.06I
	S^2	.222	.059	.070	.081	.111	.129		
43C	$\bar{X}$	.487	.487	.462	.437	.475	.462	0.42	1.79I
	S^2	.253	.253	.252	.249	.253	.252		
22E	$\bar{X}$	.637	.850	.887	.862	.875	.862	19.90**	2.05I
	S^2	.234	.129	.101	.120	.111	.120		
23E	$\bar{X}$	.762	.912	.925	.912	.925	.900	51.52**	1.90I
	S^2	.183	.081	.070	.081	.070	.091		
24E	$\bar{X}$	.837	.950	.900	.912	.912	.862	82.21**	1.78*
	S^2	.138	.048	.091	.081	.081	.120		
44C	$\bar{X}$	.462	.487	.487	.512	.500	.512	0.24	2.16
	S^2	.252	.253	.253	.253	.253	.253		

*$F_{(6,74)}$ = 2.59 at P = .025; D_L=1.83 and D_U=2.16 at P = .05.

**$F_{(6,74)}$ = 3.08 at P = .01.

I D-value was inconclusive.

Table 4.3 (cont'd.)

Group		Time in Days 1	2	3	4	F	D
25E	$\bar{X}$	.550	.550	.550	.562	0.46	2.24
	S^2	.251	.251	.251	.249		
26E	$\bar{X}$	.662	.662	.650	.650	3.04*	2.21
	S^2	.226	.226	.230	.230		
27E	$\bar{X}$	.662	.737	.775	.775	10.49**	2.31
	S^2	.226	.196	.177	.177		
45C	$\bar{X}$	.462	.525	.487	.525	1.10	2.32
	S^2	.252	.253	.253	.253		
28E	$\bar{X}$	.562	.662	.675	.675	3.74**	2.08I
	S^2	.249	.226	.222	.222		
29E	$\bar{X}$	.750	.825	.875	.837	25.35**	2.37
	S^2	.190	.146	.111	.138		
30E	$\bar{X}$	.787	.837	.875	.862	24.76**	2.14
	S^2	.169	.138	.111	.120		
46C	$\bar{X}$	.500	.462	.475	.475	0.37	2.14
	S^2	.253	.252	.253	.253		
31E	$\bar{X}$	.812	.850	.800	.812	27.03**	1.96I
	S^2	.154	.129	.162	.154		
32E	$\bar{X}$	.775	.887	.912	.875	43.59**	2.12
	S^2	.177	.101	.081	.111		
33E	$\bar{X}$	.850	.950	.937	.925	81.84**	2.20
	S^2	.129	.048	.059	.070		
47C	$\bar{X}$	.450	.500	.487	.462	0.66	2.31
	S^2	.251	.253	.253	.252		
34E	$\bar{X}$	.925	.975	.987	.975	424.33**	2.33
	S^2	.070	.025	.012	.025		
35E	$\bar{X}$	.875	.975	.987	.962	674.79**	2.47
	S^2	.111	.025	.012	.037		
36E	$\bar{X}$	.650	.762	.750	.737	7.54**	2.24
	S^2	.230	.183	.190	.196		
48C	$\bar{X}$	.450	.437	.462	.475	1.25	1.96I
	S^2	.251	.249	.252	.253		

*$F_{(4,76)} = 2.96$ at P = .025; D_L=1.89 and D_U=2.10 at P = .05.

**$F_{(4,76)} = 3.59$ at P = .01.

ID-value was inconclusive.

that we would not otherwise have. This will be discussed later, but an example will help now. If we look at the estimated variances in Table 4.3, we find that the variances decrease as degree of conditioning increases, that is, groups 2E, 5E, 8E, and 11E. In Table 4.4 the Two-Sample test comparing 2E to 5E gave an F2-value of 1.84 and the null hypothesis was not rejected. However, Box's test says that the variances are heterogeneous indicating that the pooled covariance matrix for this test was not the best estimate, and that we may have accepted a false null hypothesis. The One-Sample comparison of groups 2E and 5E yielded an F1-value of 3.44 which is significant at the .01 level. Therefore, the heterogeneous variance was responsible for originally not rejecting the hypothesis. We now know that the variances between the two groups are different and that it is greater in the group (2E) responding to the lowest degree of conditioning. These facts tell us that larvae in the two groups are not responding in the same way to different degrees of conditioning even if their mean response is consistent from one experiment to another. It appears that response to low conditioning is much more variable.

The One-Sample procedure, therefore, is being used only as a tool to help dissect the data. The Two-Sample procedure is still the legitimate one and will be used for the final conclusions on the experiments. It should be pointed out, however, that the One-Sample procedure does not alter any of the conclusions, it only reinforces them and adds to our understanding of what is happening biologically.

Since both One- and Two-Sample procedures were used, some experimental groups were multiply compared as many as 20 times which

necessitated a reconsideration of the appropriate α-level for hypothesis testing. All tables in this paper show the critical values for α-levels of .025, .01, and .001. Any rejection of the null hypothesis with the Two-Sample test, even at the .025 level, is valid since it is difficult to reject when the variances are heterogeneous. Since the One-Sample test has eliminated the source of the greatest variability, any rejection at the .025 level must be cautiously interpreted because homogeneous variances make it easier to reject the null hypothesis.

Pilot Experiment

One of the reasons Galleria larvae were selected for these studies is that they produce discrete silken tunnels in the food as well as deposit copious amounts of frass. This is an obvious form of physical homotypic conditioning, although there may be chemical conditioning as well. However, it was first necessary to determine if such conditioning would elicit a response from conspecifics before the design in Figure 4.2 was run.

To determine whether Galleria larvae respond to homotypic conditioning an 80 dish experiment was set up with CAT = 7, 6, 7. Data was collected every 24 hours, and the mean response vector is shown in Figure 4.3 as compared to an expected vector whose entries are all 0.5. The response vector is significantly different from random with F = 20.80, where $F_{(14,\ 66)} = 3.18$ at P = 0.001. The curve has also been divided into "Larval" Phase and "Wandering" Phase.

As a result of this pilot study it is obvious that larvae respond to conditioning, in this case they are attracted to it. When

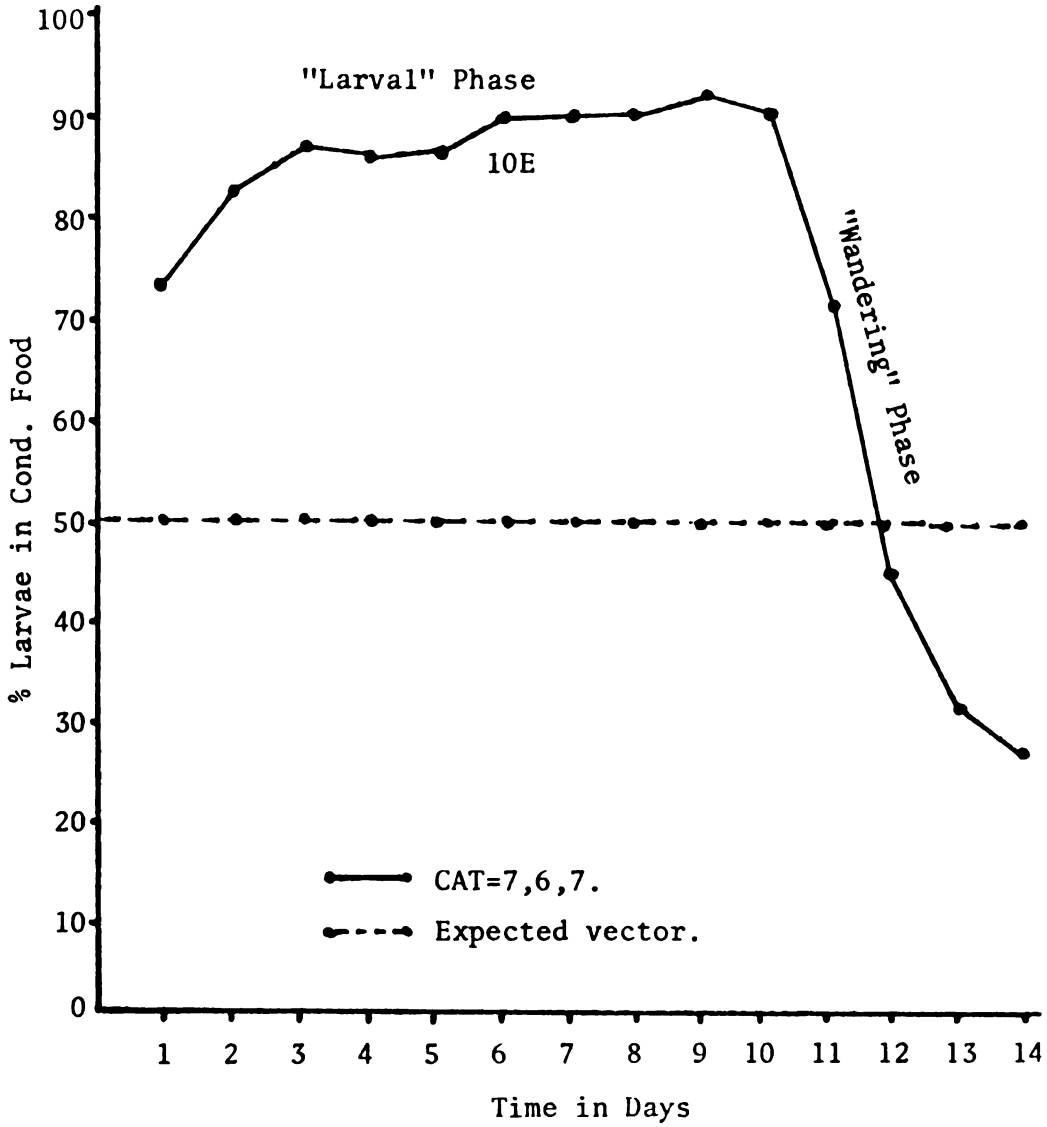


Figure 4.3
Pilot experiment to see if Galleria larvae respond to biologically conditioned food. Note that after 16 days of age the response begins declining during which time the larvae are apparently not exhibiting a preference for either conditioned or non-conditioned food.

the larvae were 16 days old (day 10 of the experiment), however, what appears to be a change in behavior occurs. The curve begins declining in what looks like repulsion to conditioning, and it would appear that the larvae are moving to the non-conditioned food lump. Observations from the experimental situation showed that larvae were all over the dishes, mostly not in any food lump. This is what the literature terms the wandering phase during which time larvae are "looking" for a place to pupate and seemingly exhibiting no preference for conditioned versus non-conditioned food. This is not an artifact of the way in which the data were calculated for the wandering phase. As previously described, a "one" was recorded if a larva was in the conditioned food lump and a "zero" if in the non-conditioned food lump. If a larva is on neither food lump, as is often the case during the wandering phase, it is effectively removed from consideration in the analysis. The fact that the response curve in Figure 4.3 drops below 50% does not mean that the larvae are now all in the non-conditioned food. What it does mean, however, is that 20% of the larva pupated in the conditioned food and the other 80% pupated in either the non-conditioned food or elsewhere in the experimental dishes. This same phenomenon occurs in every group in Table 4.2, regardless of degree of conditioning or age of test larvae. It would appear, then, that we are dealing with a behaviorally different organism once it reaches 16 days of age and there is no a priori basis upon which to calculate an expected vector for the wandering phase. For these reasons analyses in this paper deal only with the test larva up through 16 days of age. The use of 16 days of larval age as the

cut-off point was determined from Figure 4.3 and from the literature which indicates that wandering begins 2 days before pupation. The mean pupation time for the larvae in this pilot experiment was 18 days and I subtracted 2 days to arrive at this 16 day figure.

Data Reduction

Aside from the fact that some data reduction had resulted from elimination of the wandering phase from analysis, it was desirable to further reduce the amount of data being analyzed for convenience sake as well as for the fact that the central memory of the computer was being exceeded with such large matrices. I decided to analyze response mean vectors consisting of only PM data points, that is, 24 hour time intervals rather than 12 hour time intervals.

In order to make this decision it is first necessary to demonstrate that daily AM responses are not different from daily PM responses within the same group. Twelve groups from Figure 4.2 were randomly selected and their AM and PM vectors analyzed with Hotelling's One- and Two-Sample T^2 Tests. The results of these comparisons are given in Table 4.2 and it will be noted that in every case the null hypothesis could not be rejected. Therefore, AM data points were eliminated from future analysis.

Results

The following description of the results has been divided into six basic parts, the first three of which directly relate to the hypotheses that response to conditioning is a function of the degree of conditioning and the age of the test larva. First, within-group

comparisons will be examined as to whether larval response to conditioning is random, attraction, or repulsion. Second, between-group comparisons of larval responses to conditioning will be analyzed as the amount of conditioning increases. Third, between-group comparisons of the effects of larval age on response to conditioning will be examined. Fourth, a brief analysis will be presented relating to initial preference to conditioning and amount of larval movement in conditioning. This analysis allows us to ask whether a larva's initial preference for conditioning is a good predictor of its long-term response and whether different degrees of conditioning alter the number of times a larva moves between the conditioned and non-conditioned food lumps. Fifth, data on whether response to conditioning can be over-ridden by varying the food source will be presented to see if larval response to conditioning is affected by the quality of food on which the conditioning is done. Finally, data on whether adult Galleria exhibit preferences to larval conditioning will be presented. This last analysis is for the purpose of discovering if larvae and adults differentially respond to larval conditioning, and if they do, to propose some possible mechanisms for the differences.

I. Within-Group Comparisons for Randomness of Larval Response to Conditioning

Hotelling's One-Sample T^2 Statistic was used to compare the response mean vector of each group in Figure 4.2 with an expected vector whose entries are all 0.5. This procedure enables determining which groups are randomly distributed and which are non-random, i.e.,

significantly attracted or repelled by conditioning. The results are shown in Table 4.3. The mean vector ($\bar{X}$) and estimated variance vector (S^2) are given for each group and should be referred to in all future comparisons in this paper. If for any two groups the reader wishes a pooled variance vector, he need only average the two vectors of concern or if the standard error is desired, it can be calculated for any entry in the variance vector as $\sqrt{S^2/N-1}$ (1.96). Table 4.3 also lists the F-values for each test as well as the D-values for serial correlation of the error terms.

The results in Table 4.3 are good indicators for the comparisons needed as tests of the other hypotheses in this paper. All control groups (37C-48C) are randomly distributed as predicted. The only experimental groups exhibiting randomness are 1E: CAT = 1, 6, 7; 2E: CAT = 1, 9, 7; 4E: CAT = 3, 6, 7; 13E: CAT = 1, 6, 11; and 25E: CAT = 1, 6, 13. In groups 1E, 13E, and 25E conditioning was by a 6 day old larva for 1 day and examination of the size of a 6 day old larva in Figure 4.1 indicates a random response might be expected. The other random responses were by groups 2E and 4E, both of which involve 7 day old test larvae. The corresponding groups for 11 day old test larvae (14E and 16E) and for 13 day old test larvae (26E and 28E) show non-random responses, indicating there may be age differences in response to the same degree of conditioning. Perhaps 7 day old larvae require the presence of more conditioning before they perceive it as different from non-conditioned. If 7 day old larvae are not responding to conditioning at the same level as 11 or 13 day

old larvae, the F-values should reflect such a trend. I will come back to this point at a later time.

Examination of the estimated variance vectors (S^2) in Table 4.3 also shows a consistent phenomenon. If the variances are compared within a test age at constant days of conditioning, i.e., 1E, 2E, and 3E, the variances get smaller as age of conditioning larva gets bigger. If the variances are compared within a test age at a constant age of conditioning larva, but across days of conditioning, i.e., 1E, 4E, 7E, and 10E, the variances get smaller as days of conditioning increase. If variances are compared for constant days of conditioning done by the same age of conditioning larva but the age of the test larva is varied, i.e., 2E, 14E, and 26E, the variances get smaller as the test larva gets older. This latter comparison can only be made for the first four days of response because the ages of test larvae are variable. These trends indicate that as degree of conditioning and/or age of test larva increases the response to conditioning becomes more stable, that is there is less variability in response. This will be further discussed later.

The results of the analysis in Table 4.3 show that the majority of responses to conditioning are non-random and in the direction of attraction, not repulsion, as shown by the mean response vectors. In the next two sections we will look at whether this attraction is the same for all degrees of conditioning and age of test larvae.

II. Between-Group Comparisons of Larval Responses to Increasing Degrees of Conditioning

There are two ways in which degree of conditioning may be viewed in the experimental design of Figure 4.2. One is to hold age of conditioning larvae constant and vary the number of days of conditioning within the three test ages. The assumption here is that the longer a larva spends in a food lump, the more conditioning it leaves. The second approach is to hold days of conditioning constant for the three test ages and vary the starting age of the conditioning larvae. The assumption is that older (bigger) larvae leave more conditioning than younger (smaller) larvae.

A. Response of Test Larvae to Conditioning Done by Varying the Number of Days of Conditioning Within Any One Age of Conditioning Larva

The F-values in Table 4.3 indicate that response of test larvae to conditioning increases as days of conditioning increase at a constant conditioning larval age. For example, when compared to an expected vector whose entries are all 0.5, the F-values for the response of 7 day old larvae to 1, 3, 5, and 7 days of conditioning done by a 6 day old larva are 0.86, 1.94, 6.82, and 23.48 respectively. Therefore, to test the hypothesis that response to conditioning increases as the number of conditioning days increases at a constant conditioning age, all between-group comparisons were made where such a situation exists.

Tables 4.4, 4.5, and 4.6 show the results of these comparisons at each test age. Presented are the comparisons made, the two F-values,

the results of Box's Test for Heterogeneity of Variances and the T^2 Test for Parallelism of two mean vectors. If two vectors are parallel it indicates that the responses are not a result of some interaction over time, but rather the larval response to two different degrees of conditioning involves the same behavior but at different levels. In other words, parallel responses indicate that the quality of behavior is the same but the quantity is different. All responses are parallel, except for 1E versus 10E and all variances are heterogeneous, except for 27E versus 36E. Figures 4.4, 4.5, and 4.6 graphically show the mean response vectors for the comparisons in the respective tables.

Examination of Figures 4.4, 4.5, and 4.6 show a definite trend to increasing response as days of conditioning increase at a constant conditioning age, regardless of age of test larvae. However, if we view these graphs in terms of age of test larvae there appears to be a difference. Older larvae respond at a higher level than do younger larvae to the same degree of conditioning (whether this is significant will be tested later). This can be seen, for example, by comparing Figure 4.4 A, B, and C for 7 day old test larvae. There is also a trend for the response vectors to become more closely grouped within a test age as age of conditioning larvae increases. For example, the three groups of curves in Figure 4.5, reading from left to right exhibit a tendency to become bunched, possibly indicating an inability on the part of the responding larva to distinguish between highly conditioned food beyond a certain level, or above a certain level they respond maximally. There may also be a threshold

Table 4.4

Results of Hotelling's One(') and Two(*) Sample comparisons of the response of 7 day old test larvae to varying amounts of conditioning (1, 3, 5, and 7 days) done by 6, 9, and 12 day old larvae.

Comparisons EV. CV.	F1	F2	Box's Test	T^2 Test for Parallelism
1E vs. 4E	2.31'	1.46	H	P
1E vs. 7E		3.26*	H	P
1E vs. 10E		6.62**	H	
4E vs. 7E	5.04'''	1.69	H	P
4E vs. 10E		3.75**	H	P
7E vs. 10E	4.97'''	1.24	H	P
2E vs. 5E	3.44''	1.73	H	P
2E vs. 8E	4.83'''	1.84	H	P
2E vs. 11E		3.35**	H	P
5E vs. 8E	1.58	0.64	H	P
5E vs. 11E		2.39*	H	P
8E vs. 11E	5.20'''	1.45	H	P
3E vs. 6E	5.30'''	2.06	H	P
3E vs. 9E		3.29**	H	P
3E vs. 12E	4.20'''	2.02	H	P
6E vs. 9E	4.49'''	1.73	H	P
6E vs. 12E	1.03	0.57	H	P
12E vs. 9E	2.96''	2.01	H	P

' $F1_{(10,70)} = 2.24$ at P = .025. * $F2_{(10,149)} = 2.15$ at P = .025.

'' $F1_{(10,70)} = 2.59$ at P = .01. ** $F2_{(10,149)} = 2.46$ at P = .01.

''' $F1_{(10,70)} = 3.48$ at P = .001. *** $F2_{(10,149)} = 3.24$ at P = .001.

EV.= expected vector and CV.= covariance matrix for the One Sample Test(').

H Heterogeneous variances ($P \leq .05$).

P Mean response vectors are parallel ($P \leq .05$).

Figure 4.4
Mean response vectors of 7 day old test larvae to varying amounts of conditioning (1, 3, 5, and 7 days) done by 6, 9, and 12 day old larvae.

A. CAT=(1,3,5,7),6,7

B. CAT=(1,3,5,7),9,7

C. CAT=(1,3,5,7),12,7

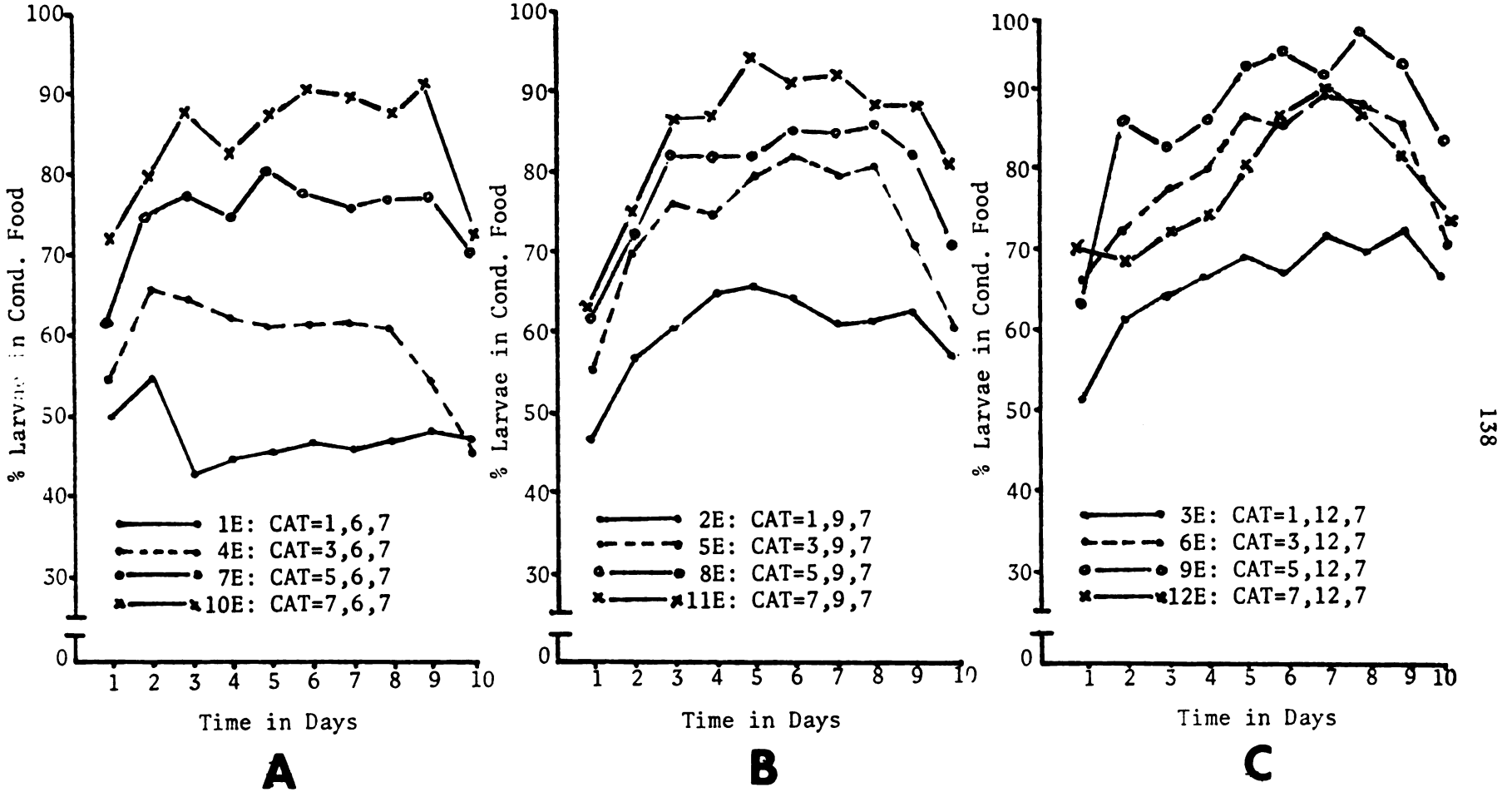

% Larvae in Cond. Food
100
90
80
70
60
50
40
30
0
1 2 3 4 5 6 7 8 9 10
Time in Days
1E: CAT=1,6,7
4E: CAT=3,6,7
7E: CAT=5,6,7
10E: CAT=7,6,7
A
% Larvae in Cond. Food
Time in Days
2E: CAT=1,9,7
5E: CAT=3,9,7
8E: CAT=5,9,7
11E: CAT=7,9,7
B
% Larvae in Cond. Food
Time in Days
3E: CAT=1,12,7
6E: CAT=3,12,7
9E: CAT=5,12,7
12E: CAT=7,12,7
C

Figure 4.4

Table 4.5

Results of Hotelling's One(') and Two(*) Sample comparisons of the response of 11 day old test larvae to varying amounts of conditioning (1, 3, 5, and 7 days) done by 6, 9, and 12 day old larvae.

Comparisons EV. CV.	F1	F2	Box's Test	T^2 Test for Parallelism
13E vs. 16E		2.95*	H	P
13E vs. 19E		4.91**	H	P
13E vs. 22E		6.00**	H	P
16E vs. 19E	5.15'''	2.29	H	P
16E vs. 22E	6.44'''	2.40	H	P
19E vs. 22E	5.07'''	0.08	H	P
14E vs. 17E	3.65''	1.19	H	P
14E vs. 20E		2.52*	H	P
14E vs. 23E		4.19**	H	P
17E vs. 20E	8.11'''	1.34	H	P
17E vs. 23E	9.94'''	1.52	H	P
20E vs. 23E	2.61'	1.17	H	P
15E vs. 18E		3.81**	H	P
15E vs. 21E		4.12**	H	P
15E vs. 24E		4.36**	H	P
18E vs. 21E	4.07''	1.86	H	P
18E vs. 24E	7.02'''	2.26	H	P
21E vs. 24E	3.29''	1.17	H	P

' $F1_{(6,74)} = 2.59$ at P = .025.
'' $F1_{(6,74)} = 3.08$ at P = .01.
''' $F1_{(6,74)} = 4.26$ at P = .001.
* $F2_{(6,153)} = 2.51$ at P = .025.
** $F2_{(6,153)} = 2.95$ at P = .01.
*** $F2_{(6,153)} = 4.03$ at P = .001.

EV.= expected vector and CV.= covariance matrix for the One Sample Test(').

H Heterogeneous variances ($P \leq .05$).

P Mean response vectors are parallel ($P \leq .05$).

Figure 4.5
Mean response vectors of 11 day old test larvae to varying amounts of conditioning (1, 3, 5, and 7 days) done by 6, 9, and 12 day old larvae.

A. CAT=(1,3,5,7),6,11

B. CAT=(1,3,5,7),9,11

C. CAT=(1,3,5,7),12,11

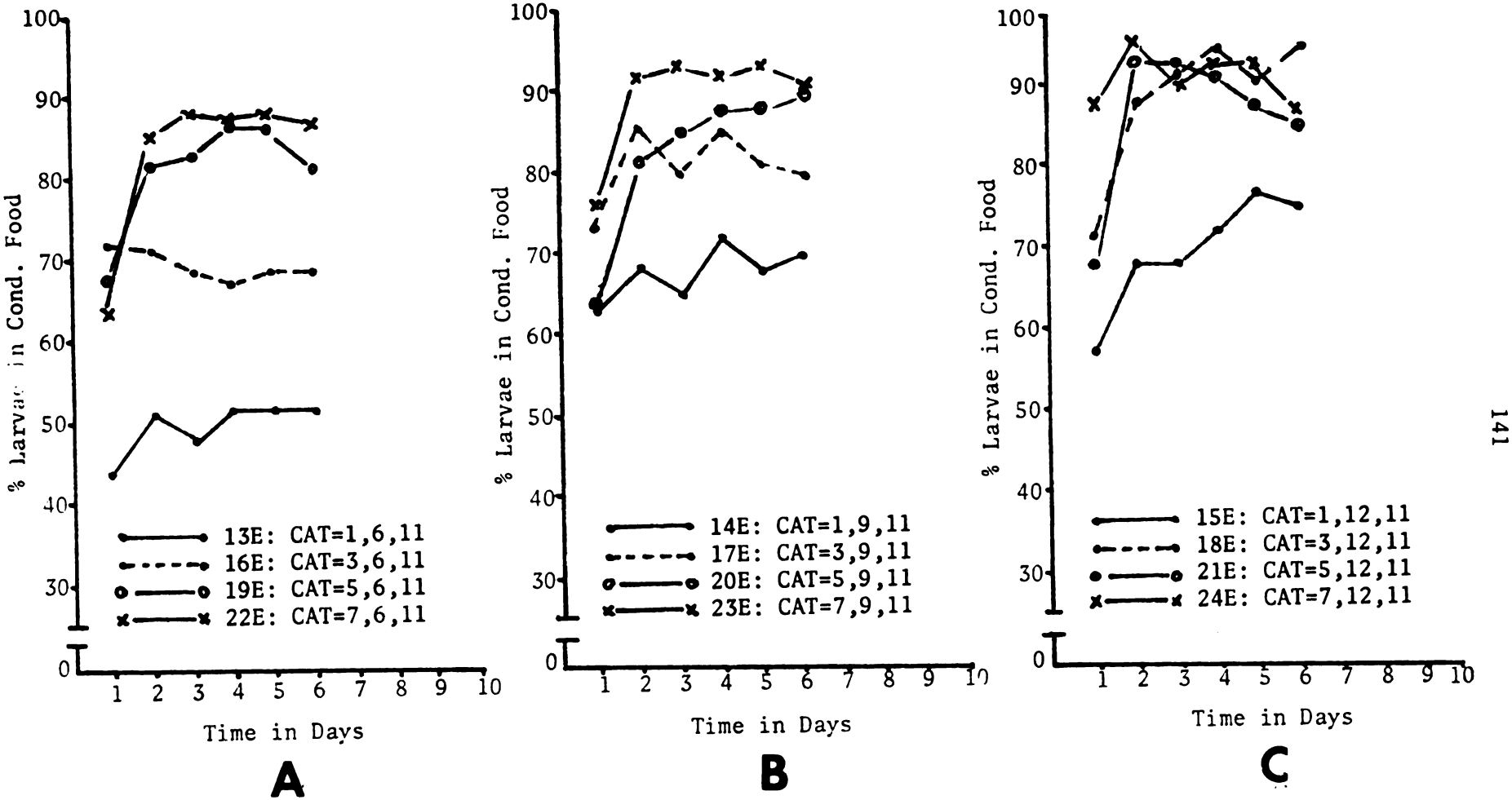

% Larvae in Cond. Food
Time in Days
13E: CAT=1,6,11
16E: CAT=3,6,11
19E: CAT=5,6,11
22E: CAT=7,6,11
A
% Larvae in Cond. Food
Time in Days
14E: CAT=1,9,11
17E: CAT=3,9,11
20E: CAT=5,9,11
23E: CAT=7,9,11
B
% Larvae in Cond. Food
Time in Days
15E: CAT=1,12,11
18E: CAT=3,12,11
21E: CAT=5,12,11
24E: CAT=7,12,11
C

Figure 4.5

Table 4.6

Results of Hotelling's One(') and Two(*) Sample comparisons of the response of 13 day old test larvae to varying amounts of conditioning (1, 3, 5, and 7 days) done by 6, 9, and 12 day old larvae.

Comparisons EV. CV.	F1	F2	Box's Test	T^2 Test for Parallelism
25E vs. 28E	1.46	0.66	H	P
25E vs. 31E		7.23***	H	P
25E vs. 34E		20.56***	H	P
28E vs. 31E		4.08**	H	P
28E vs. 34E		12.97***	H	P
31E vs. 34E		5.04***	H	P
26E vs. 29E		3.29*	H	P
26E vs. 32E		4.50**	H	P
26E vs. 35E		9.37***	H	P
29E vs. 32E	0.99	0.32	H	P
29E vs. 36E		3.01*	H	P
32E vs. 35E	17.53'''	1.73	H	P
27E vs. 30E	2.33	0.92	H	P
27E vs. 33E		4.39**	H	P
36E vs. 27E		1.25		P
30E vs. 33E	11.13'''	1.82	H	P
30E vs. 36E	3.91''	2.13	H	P
33E vs. 36E		3.85**	H	P

' $F1_{(4,76)} = 2.96$ at P = .025. * $F2_{(4,155)} = 2.88$ at P = .025.

'' $F1_{(4,76)} = 3.59$ at P = .01. ** $F2_{(4,155)} = 3.47$ at P = .01.

''' $F1_{(4,76)} = 5.18$ at P = .001. *** $F2_{(4,155)} = 4.94$ at P = .001.

EV.= expected vector and CV.= covariance matrix for the One Sample Test(').

H Heterogeneous variances ($P \leq .05$).

P Mean response vectors are parallel ($P \leq .05$).

Figure 4.6
Mean response vectors of 13 day old test larvae to varying amounts of conditioning (1, 3, 5, and 7 days) done by 6, 9, and 12 day old larvae.

A. CAT=(1,3,5,7),6,13

B. CAT=(1,3,5,7),9,13

C. CAT=(1,3,5,7),12,13

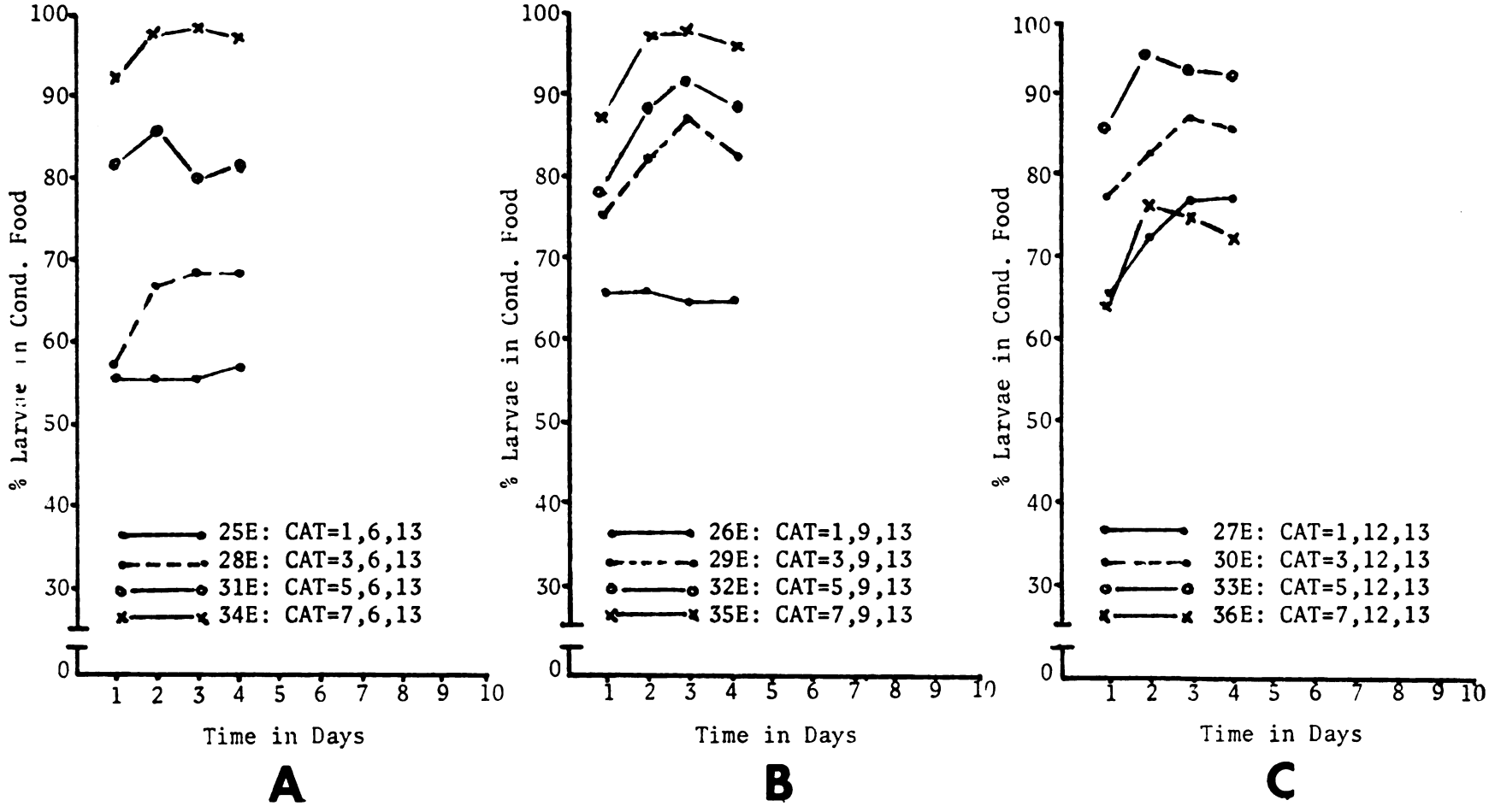

% Larvae in Cond. Food
100
90
80
70
60
50
40
30
0
1 2 3 4 5 6 7 8 9 10
Time in Days
25E: CAT=1,6,13
28E: CAT=3,6,13
31E: CAT=5,6,13
34E: CAT=7,6,13
A
% Larvae in Cond. Food
Time in Days
26E: CAT=1,9,13
29E: CAT=3,9,13
32E: CAT=5,9,13
35E: CAT=7,9,13
B
% Larvae in Cond. Food
Time in Days
27E: CAT=1,12,13
30E: CAT=3,12,13
33E: CAT=5,12,13
36E: CAT=7,12,13
C

Figure 4.6

effect where any greater amount of conditioning beyond a certain level does not elicit any greater response of the test larvae.

Although the curves in Figures 4.4, 4.5, and 4.6 exhibit a trend to increasing response as days of conditioning increase for a particular conditioning age, the F2-values for the Two-Sample test in Tables 4.4, 4.5, and 4.6 demonstrate that not all such responses are significantly different. However, within any group of responses at a constant conditioning age, but at varying days of conditioning, we would expect increasing responses as days of conditioning increase. Examination of Figures 4.4, 4.5, and 4.6 A, B, and C shows this to be the case and the F2-values confirm this hypothesis. If we look across any one figure, i.e., 4.6 A, B, and C, a tendency for the curves to bunch is shown. If the curves are grouping about a common response level then there should be fewer significant F2-values in the tables as age of conditioning larvae increases. This hypothesis holds for all except Figure 4.5C where there are more significant differences than would be predicted. This may be a result of the fact that Figures 4.4C and 4.6C have a group behaving in an unpredictable manner, namely 12E and 36E, whereas as the comparable group, 24E, in Figure 4.5C is behaving normally.

Groups 12E and 36E did not respond as predicted. Group 12E, Figure 4.4C, should have been the highest response vector yet it is in the middle, and group 36E, Figure 4.6C, should have been the highest but is the lowest response vector. The conditioning for these groups was done by a 12 day old larva for 7 days. However, after 7 days an initially 12 day old larva is 19 days old and has pupated. Observations

on the experimental dishes revealed that the silken tunnels normally spun on the food had been chewed up and possibly re-absorbed as these conditioning larvae pupated. Assuming response to conditioning to be a response to these tunnels, then in groups 12E and 36E there would be less conditioning to respond to. Other explanations are possible as well. For example, wandering and pupating larvae may secrete substances that repel the test larvae. Group 24E Figure 4.5C, was also conditioned by a 12 day old larva for 7 days, yet the response of 11 day old larvae to this conditioning was as predicted. There is no apparent explanation for this except that the discriminating ability of 11 day old larvae may be greater than either 7 or 13 day old test larvae. However, the fact that 11 day old larvae respond as predicted tends to negate any hypothesis about wandering larvae secreting some sort of repulsive substance, unless repulsion is age dependent.

If the F1-values for the One-Sample test are examined we find more rejections of the null hypothesis than with the Two-Sample test. However, the trends indicated by the Two-Sample test are now significant with the One-Sample test. This demonstrates that the reason for not rejecting many of the null hypotheses is that there is heterogeneous variance between groups. In any comparisons which are not significantly different with the One-Sample procedure we can be sure that heterogeneous variances are not the reason for failure to reject the null hypothesis.

B. Response of Test Larvae to Conditioning Done by Varying Ages of Conditioning Larvae at Constant Days of Conditioning

Table 4.3 indicates that response of test larvae to conditioning done by varying ages of larvae at constant days of conditioning increases as age of conditioning larvae increases. This probably means that either older (bigger) larvae leave more conditioning than younger (smaller) larvae in the same unit of time, or that different ages of larvae secrete different substances. The F-values in Table 4.3 exhibit an increasing tendency under such conditions. For example, in the response of an 11 day old larva to 1 day of conditioning by a 6, 9, or 12 day old larva, the F-values are 0.49, 4.35, and 7.07, or the F-values for the response of a 7 day old larva to 3 days of conditioning by a 6, 9, or 12 day old larva are 1.94, 8.50, and 23.97 respectively. To test the hypothesis that response to conditioning increases as the age of the conditioning larva increases at constant days of conditioning the comparisons in Tables 4.7, 4.8, and 4.9 were made and graphed in Figures 4.7, 4.8, and 4.9. All variances are heterogeneous, and except for comparisons 1E versus 3E and 16E versus 18E, all response vectors are parallel.

The results of these comparisons show the same trends in response as for varying days of conditioning within any one age of conditioning larvae. That is, as age of conditioning larva increases the response vectors increase. For example, the following trends are evident from Figure 4.7, and similar trends are found in Figures 4.8 and 4.9.

Table 4.7

Results of Hotelling's One(') and Two(*) Sample comparisons of the response of 7 day old test larvae to conditioning done by 6, 9, and 12 day old larvae as days of conditioning are held constant at 1, 3, 5, and 7 days.

Comparisons EV. CV.	F1	F2	Box's Test	T^2 Test for Parallelism
1E vs. 2E	3.62	1.39	H	P
1E vs. 3E		2.23*	H	
2E vs. 3E	0.88	0.37	H	P
4E vs. 5E	3.66'''	1.27	H	P
4E vs. 6E		3.07**	H	P
5E vs. 6E	3.01''	1.04	H	P
7E vs. 8E	1.57	0.58	H	P
7E vs. 9E		2.21*	H	P
8E vs. 9E	5.92'''	1.36	H	P
10E vs. 11E	4.20'''	1.78	H	P
10E vs. 12E	2.51'	1.46	H	P
12E vs. 11E	2.48'	1.83	H	P

' $F1_{(10,70)} = 2.24$ at P = .025.
'' $F1_{(10,70)} = 2.59$ at P = .01.
''' $F1_{(10,70)} = 3.48$ at P = .001.
* $F2_{(10,149)} = 2.15$ at P = .025.
** $F2_{(10,149)} = 2.46$ at P = .01.
*** $F2_{(10,149)} = 3.24$ at P = .001.

EV.= expected vector and CV.= covariance matrix for the One Sample Test(').

H Heterogeneous variances ($P \leq .05$).

P Mean response vectors are parallel ($P \leq .05$).

Figure 4.7
Mean response vectors of 7 day old test larvae to conditioning done by 6, 9, and 12 day old larvae as days of conditioning are held constant at 1, 3, 5, or 7 days.

A. CAT=1,(6,9,12),7

B. CAT=3,(6,9,12),7

C. CAT=5,(6,9,12),7

D. CAT=7,(6,9,12),7

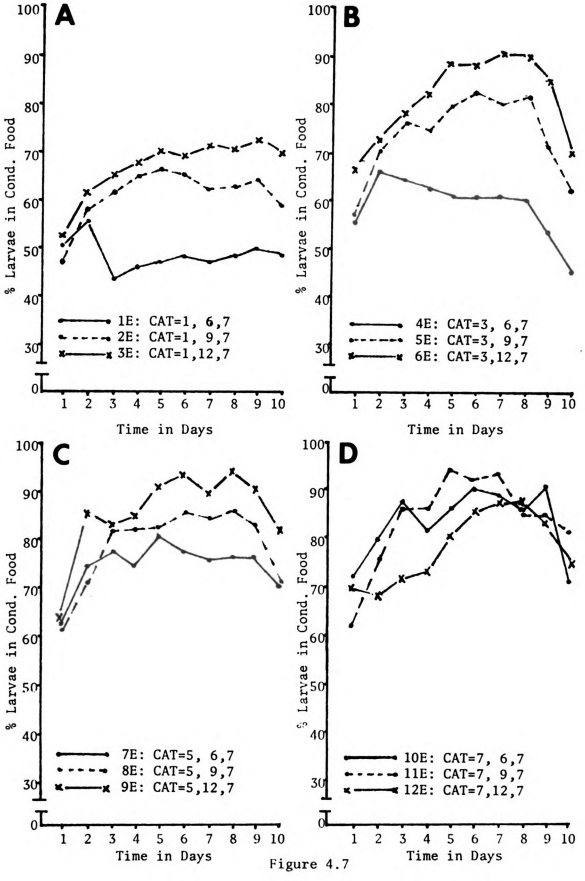

A
% Larvae in Cond. Food
Time in Days
1E: CAT=1, 6,7
2E: CAT=1, 9,7
3E: CAT=1,12,7
B
% Larvae in Cond. Food
Time in Days
4E: CAT=3, 6,7
5E: CAT=3, 9,7
6E: CAT=3,12,7
C
% Larvae in Cond. Food
Time in Days
7E: CAT=5, 6,7
8E: CAT=5, 9,7
9E: CAT=5,12,7
D
% Larvae in Cond. Food
Time in Days
10E: CAT=7, 6,7
11E: CAT=7, 9,7
12E: CAT=7,12,7

Figure 4.7

Table 4.8

Results of Hotelling's One(') and Two(*) Sample comparisons of the response of 11 day old test larvae to conditioning done by 6, 9, and 12 day old larvae as days of conditioning are held constant at 1, 3, 5, or 7 days.

Comparisons EV. CV.	F1	F2	Box's Test	T^2 Test for Parallelism
13E vs. 14E	4.79'''	2.13	H	P
13E vs. 15E		2.84*	H	P
14E vs. 15E	4.25''	2.33	H	P
16E vs. 17E	5.28'''	2.48	H	P
16E vs. 18E		4.18***	H	
17E vs. 18E	8.11'''	1.84	H	P
19E vs. 20E	1.79	0.68	H	P
19E vs. 21E	7.49'''	1.37	H	P
20E vs. 21E	9.71'''	1.90	H	P
22E vs. 23E	1.49	0.59	H	P
22E vs. 24E	7.44'''	2.34	H	P
24E vs. 23E	1.90	1.09	H	P

' $F1_{(6,74)} = 2.59$ at P = .025. * $F2_{(6,153)} = 2.51$ at P = .025.

'' $F1_{(6,74)} = 3.08$ at P = .01. ** $F2_{(6,153)} = 2.95$ at P = .01.

''' $F1_{(6,74)} = 4.26$ at P = .001. *** $F2_{(6,153)} = 4.03$ at P = .001.

EV.= expected vector and CV.= covariance matrix for the One Sample Test(').

H Heterogeneous variances ($P \leq .05$).

P Mean response vectors are parallel ($P \leq .05$).

Figure 4.8
Mean response vectors of 11 day old test larvae to conditioning done by 6, 9, and 12 day old larvae as days of conditioning are held constant at 1, 3, 5, or 7 days.

A. CAT=1,(6,9,12),11

B. CAT=3,(6,9,12),11

C. CAT=5,(6,9,12),11

D. CAT=7,(6,9,12),11

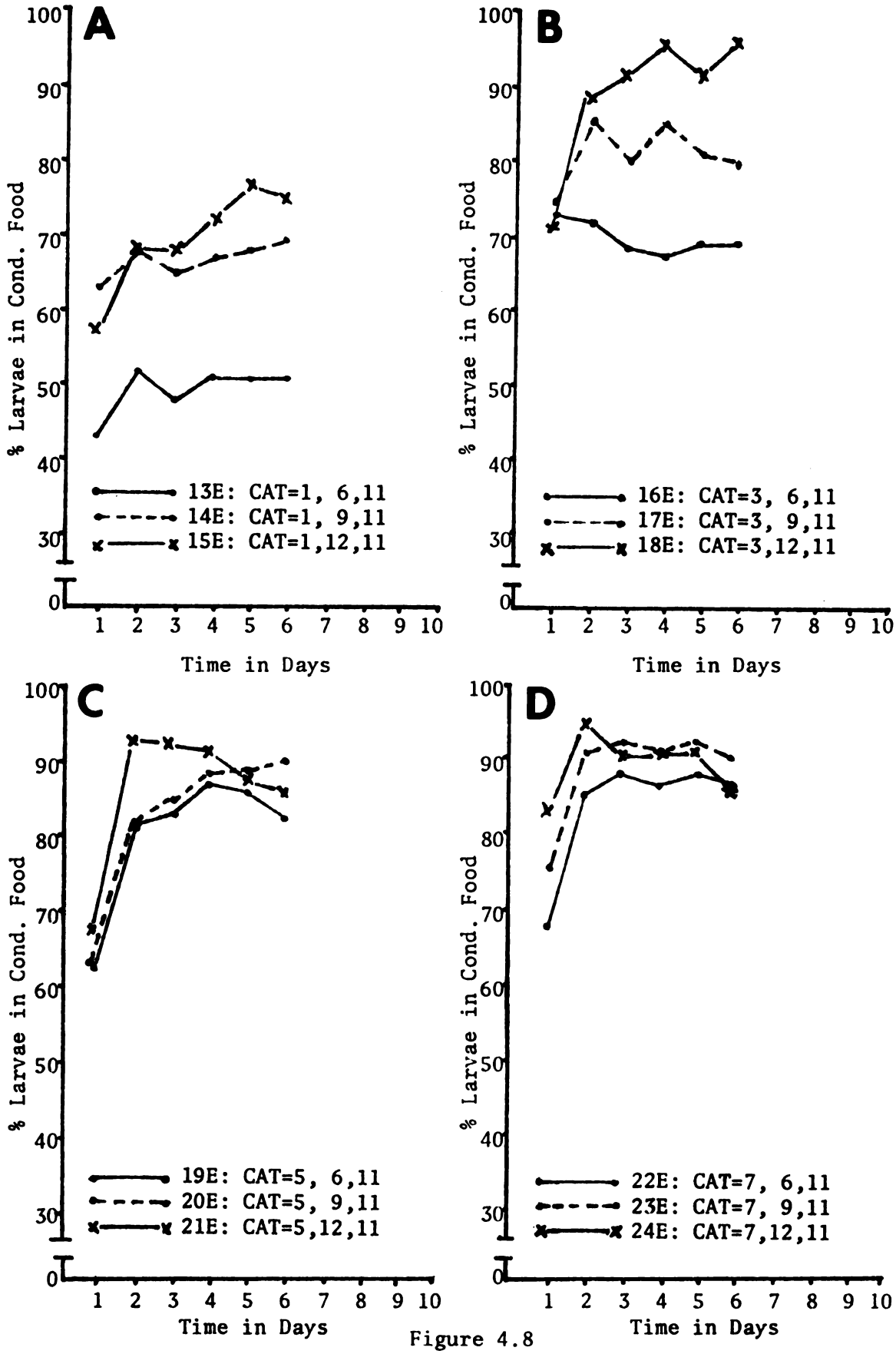

A
B
C
D
% Larvae in Cond. Food
Time in Days
100
90
80
70
60
50
40
30
0
1 2 3 4 5 6 7 8 9 10
13E: CAT=1, 6,11
14E: CAT=1, 9,11
15E: CAT=1,12,11
16E: CAT=3, 6,11
17E: CAT=3, 9,11
18E: CAT=3,12,11
19E: CAT=5, 6,11
20E: CAT=5, 9,11
21E: CAT=5,12,11
22E: CAT=7, 6,11
23E: CAT=7, 9,11
24E: CAT=7,12,11

Figure 4.8

Table 4.9

Results of Hotelling's One(') and Two(*) Sample comparisons of the response of 13 day old test larvae to conditioning done by 6, 9, and 12 day old larvae as days of conditioning are held constant at 1, 3, 5, or 7 days.

Comparisons EV. CV.	F1	F2	Box's Test	T^2 Test for Parallelism
25E vs. 26E	4.93''	0.91	H	P
25E vs. 27E	6.79'''	2.86	H	P
26E vs. 27E	4.34''	1.78	H	P
28E vs. 29E		3.35**	H	P
28E vs. 30E		3.46*	H	P
29E vs. 30E	1.33	0.31	H	P
31E vs. 32E	7.61'''	2.14	H	P
31E vs. 33E	6.97	1.68	H	P
32E vs. 33E	3.81''	1.12	H	P
35E vs. 34E	0.63	0.32	H	P
36E vs. 34E		8.52***	H	P
36E vs. 35E		6.75***	H	P

' $F1_{(4,76)} = 2.88$ at P = .025.
'' $F1_{(4,76)} = 3.59$ at P = .01.
''' $F1_{(4,76)} = 5.18$ at P = .001.
* $F2_{(4,155)} = 2.88$ at P = .025.
** $F2_{(4,155)} = 3.47$ at P = .01.
*** $F2_{(4,155)} = 4.94$ at P = .001.

EV.= expected vector and CV.= covariance matrix for the One Sample Test(').

H Heterogeneous variances ($P \leq .05$).

P Mean response vectors are parallel ($P \leq .05$).

Figure 4.9
Mean response vectors of 13 day old test larvae to conditioning done by 6, 9, and 12 day old larvae as days of conditioning are held constant at 1, 3, 5, or 7 days.

A. CAT=1,(6,9,12),13

B. CAT=3,(6,9,12),13

C. CAT=5,(6,9,12),13

D. CAT=7,(6,9,12),13

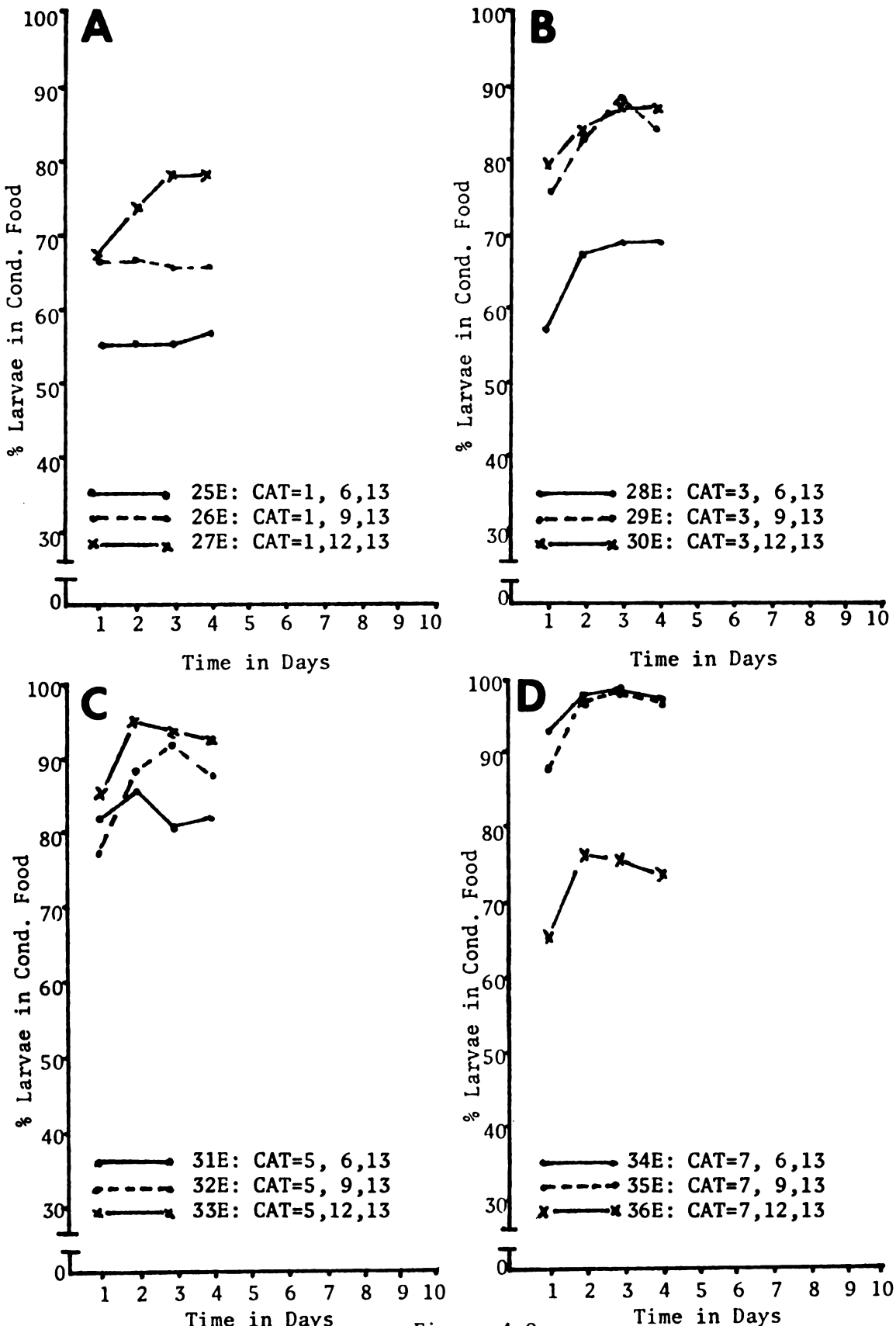

A
% Larvae in Cond. Food
100
90
80
70
60
50
40
30
0
1 2 3 4 5 6 7 8 9 10
Time in Days
25E: CAT=1, 6,13
26E: CAT=1, 9,13
27E: CAT=1,12,13
B
% Larvae in Cond. Food
Time in Days
28E: CAT=3, 6,13
29E: CAT=3, 9,13
30E: CAT=3,12,13
C
% Larvae in Cond. Food
Time in Days
31E: CAT=5, 6,13
32E: CAT=5, 9,13
33E: CAT=5,12,13
D
% Larvae in Cond. Food
Time in Days
34E: CAT=7, 6,13
35E: CAT=7, 9,13
36E: CAT=7,12,13

Figure 4.9

1. Within any particular day of conditioning and age of test larva, response to conditioning increases as the age of the conditioning larva increases (Figure 4.7A, B, C, or D).
2. As days of conditioning increase (Figure 4.7A, B, C, and D) the response to conditioning by a particular age of test larva increases. That is, the response of a 7 day old larva is greater to 7 days of conditioning by a 9 day old larva than it is to 1 day of conditioning by a 9 day old larva.
3. As days of conditioning increase (Figure 4.7A, B, C, and D) there is less discrimination in response to conditioning by the 3 ages of test larvae. For example, the response vectors in Figure 4.7D are at a higher level and more bunched than in Figure 4.7A.

Even though these trends are evident in Figures 4.7, 4.8, and 4.9, the F-values in Tables 4.7, 4.8, and 4.9 indicate that there are only a few significant differences in response to conditioning done by the three ages of conditioning larvae within a constant test age and constant days of conditioning. This may be a function of the ages of conditioning larvae chosen for these experiments, i.e., 6, 9, and 12 days old. If the ages had been more spread out, i.e., 5, 10, and 15 days old, the significant differences should have been more numerous.

However, this lack of significant differences in response to conditioning by different aged larva is more probably a function of heterogeneous variances. The F1-values in Tables 4.7, 4.8, and 4.9 exhibit many more significant differences among comparisons.

The problem with the response vectors for groups 12E, 24E, and 36E is again evident.

III. Between-Group Comparisons of the Effects of Larval Age on Response to Conditioning

Table 4.3 indicates that there may be age differences relating to larval response to homotypic conditioning. If such differences exist, the F-values for the response to the same degree of conditioning by the same aged conditioning larva should vary, i.e., increase, as age of test larvae increases. For example, in Table 4.3 the F-values for response of 7, 11, and 13 day old test larvae to 3 days of conditioning done by a 9 day old conditioning larva (groups 5E, 17E, and 29E) are 8.59, 17.70, and 25.35, respectively. This indicates that older larvae respond at a higher level than younger larvae to the same degree of conditioning.

Since the response vectors for 7, 11, and 13 day old larvae are not compatible time-wise, only the first 4 days of response were used for each comparison. The results of these comparisons are shown in Tables 4.10, 4.11, and 4.12 and the corresponding Figures 4.10, 4.11, and 4.12. All comparisons exhibit heterogeneous variances and all comparisons are parallel.

With the Two-Sample comparison (F2-values) only significant differences in response due to age show up when there are 7 days of conditioning done by 6, 9, or 12 day old larvae. Groups 12E, 24E, and 36E have been ignored because they are atypical, as previously discussed. Therefore, age differences prevail when CAT=7,6(7,13) [comparison 10E versus 34E, Table 4.10, Figure 4.10D], CAT=7,6(11,13)

Table 4.10

Results of Hotelling's One(') and Two(*) Sample comparisons for age differences in response to conditioning. The responses of test larvae (7, 11, and 13 days old) to 1, 3, 5, or 7 days of conditioning done by a 6 day old larva are tested.

Comparisons EV.	CV.	F1	F2	Box's Test	T^2 Test for Parallelism
13E vs.	1E	8.33'''	1.26	H	P
1E vs.	25E	12.19'''	1.82	H	P
13E vs.	25E	1.55	0.72	H	P
4E vs.	16E	3.00'	1.36	H	P
4E vs.	28E	0.51	0.13	H	P
28E vs.	16E	2.53	1.24	H	P
7E vs.	19E	3.29'	1.35	H	P
7E vs.	31E	7.97'''	2.77	H	P
19E vs.	31E	5.85'''	2.32	H	P
10E vs.	22E	2.16	0.89	H	P
10E vs.	34E		4.95**	H	P
22E vs.	34E		6.35**	H	P

' $F1_{(4,76)} = 2.96$ at P = .025. * $F2_{(4,155)} = 2.88$ at P = .025.
'' $F1_{(4,76)} = 3.59$ at P = .01. ** $F2_{(4,155)} = 3.47$ at P = .01.
''' $F1_{(4,76)} = 5.18$ at P = .001. *** $F2_{(4,155)} = 4.94$ at P = .001.

EV.= expected vector and CV.= covariance matrix for the One Sample Test(').

H Heterogeneous variances ($p \leq .05$).

P Mean response vectors are parallel ($P \leq .05$).

Figure 4.10
Mean response vectors of 7, 11, and 13 day old test larvae to 1, 3, 5, or 7 days of conditioning done by a 6 day old larva.

A. CAT=1,6,(7,11,13)

B. CAT=3,6,(7,11,13)

C. CAT=5,6,(7,11,13)

D. CAT=7,6,(7,11,13)

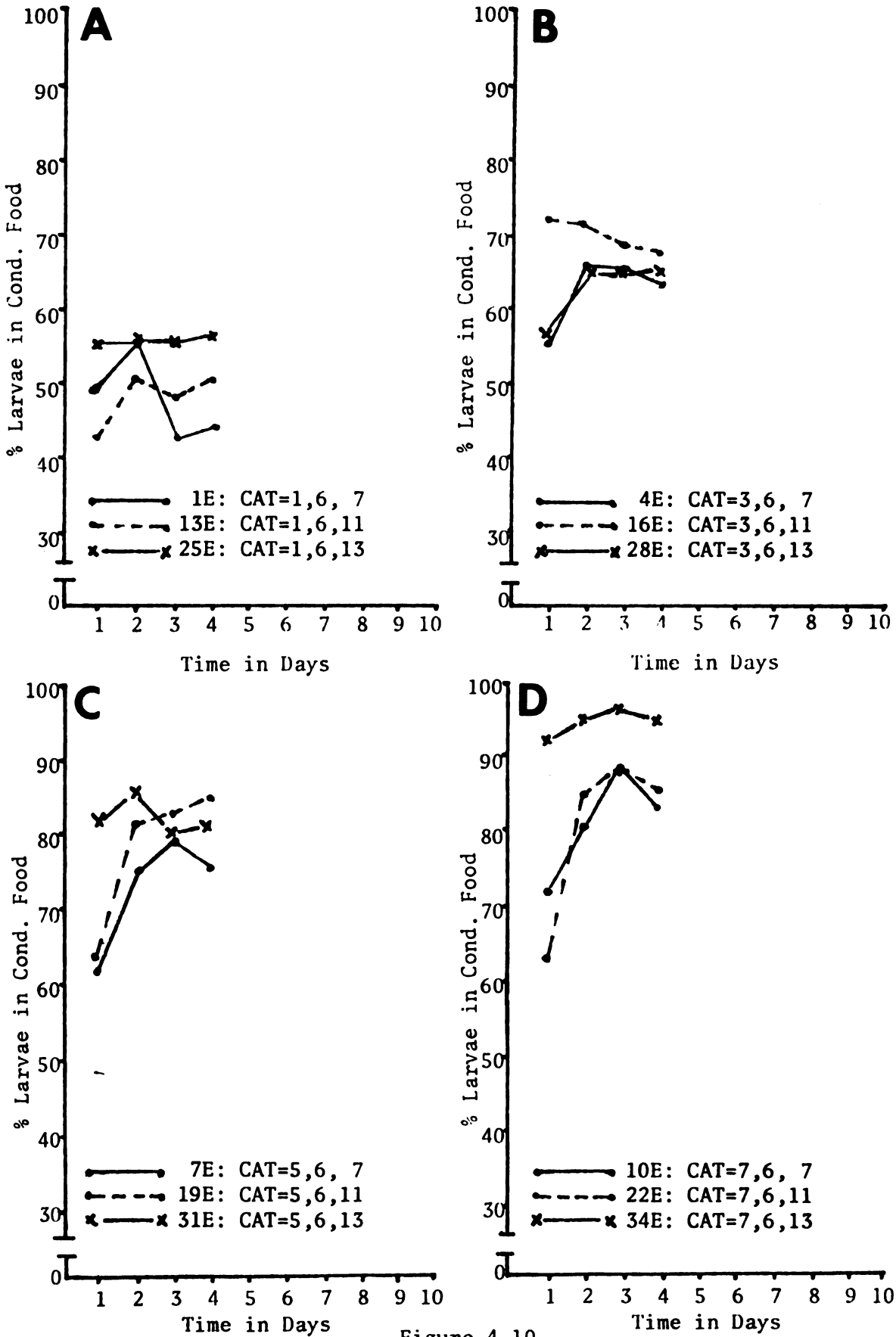

A
% Larvae in Cond. Food
100
90
80
70
60
50
40
30
0
1E: CAT=1,6, 7
13E: CAT=1,6,11
25E: CAT=1,6,13
1 2 3 4 5 6 7 8 9 10
Time in Days
B
% Larvae in Cond. Food
4E: CAT=3,6, 7
16E: CAT=3,6,11
28E: CAT=3,6,13
Time in Days
C
% Larvae in Cond. Food
7E: CAT=5,6, 7
19E: CAT=5,6,11
31E: CAT=5,6,13
Time in Days
D
% Larvae in Cond. Food
10E: CAT=7,6, 7
22E: CAT=7,6,11
34E: CAT=7,6,13
Time in Days

Figure 4.10

Table 4.11

Results of Hotelling's One(') and Two(*) Sample comparisons for age differences in response to conditioning. The responses of test larvae (7, 11, and 13 days old) to 1, 3, 5, or 7 days of conditioning by a 9 day old larva are tested.

Comparisons EV.	CV.	F1	F2	Box's Test	T^2 Test for Parallelism
2E vs.	14E	4.06''	1.72	H	P
2E vs.	26E	6.52'''	1.90	H	P
26E vs.	14	4.71''	1.19	H	P
5E vs.	17E	5.82'''	2.71	H	P
5E vs.	29E	5.24'''	1.96	H	P
17E vs.	19E	5.96'''	1.39	H	P
8E vs.	20E	1.70	0.62	H	P
8E vs.	32E	6.15'''	1.96	H	P
20E vs.	32E	2.64	1.57	H	P
11E vs.	23E	6.52'''	2.05	H	P
11E vs.	35E		5.47***	H	P
23E vs.	35E	9.59'''	1.30		

' $F1_{(4,76)} = 2.96$ at P = .025. * $F2_{(4,155)} = 2.88$ at P = .025.

'' $F1_{(4,76)} = 3.59$ at P = .01. ** $F2_{(4,155)} = 3.47$ at P - .01.

''' $F1_{(4,76)} = 5.18$ at P = .001. *** $F2_{(4,155)} = 4.94$ at P = .001.

EV.= expected vector and CV.= covariance matrix for the One Sample Test(').

H Heterogeneous variances ($P \leq .05$).

P Mean response vectors are parallel ($P \leq .05$).

Figure 4.11
Mean response vectors of 7, 11, and 13 day old test larvae to 1, 3, 5, or 7 days of conditioning done by a 9 day old larva.

A. CAT=1,9,(7,11,13)

B. CAT=3,9,(7,11,13)

C. CAT=5,9,(7,11,13)

D. CAT=7,9,(7,11,13)

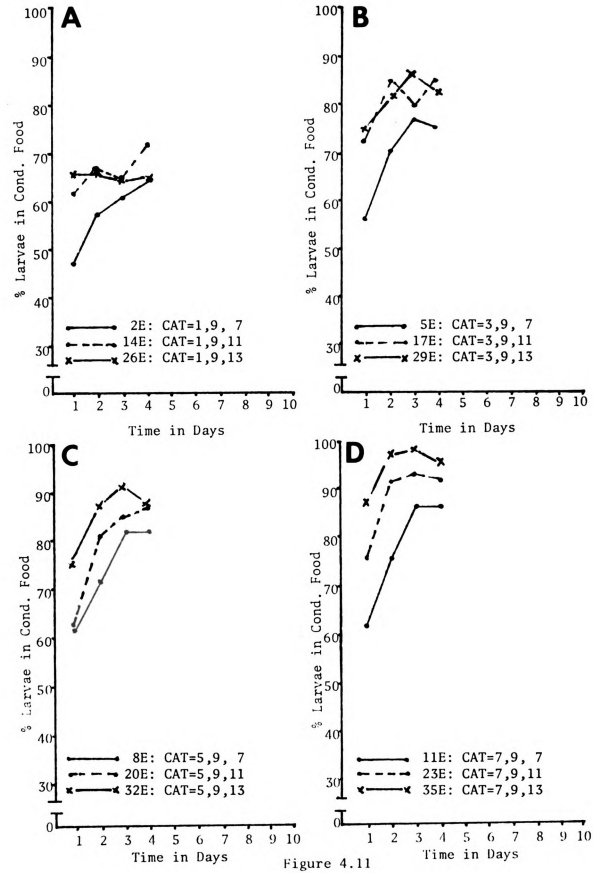

A
% Larvae in Cond. Food
2E: CAT=1,9, 7
14E: CAT=1,9,11
26E: CAT=1,9,13
Time in Days
B
% Larvae in Cond. Food
5E: CAT=3,9, 7
17E: CAT=3,9,11
29E: CAT=3,9,13
Time in Days
C
% Larvae in Cond. Food
8E: CAT=5,9, 7
20E: CAT=5,9,11
32E: CAT=5,9,13
Time in Days
D
% Larvae in Cond. Food
11E: CAT=7,9, 7
23E: CAT=7,9,11
35E: CAT=7,9,13
Time in Days

Figure 4.11

Table 4.12

Results of Hotelling's One(') and Two(*) Sample comparisons for age differences in response to conditioning. The responses of test larvae (7, 11, and 13 days old) to 1, 3, 5, and 7 days of conditioning by a 12 day old larva are tested.

Comparisons EV. CV.	F1	F2	Box's Test	T^2 Test for Parallelism
3E vs. 15E	0.63	0.33	H	P
3E vs. 27E	2.44	1.20	H	P
15E vs. 27E	1.73	0.73	H	P
6E vs. 18E	9.46'''	2.20	H	P
6E vs. 30E	4.04''	1.36	H	P
30E vs. 18E	6.47'''	2.34	H	P
9E vs. 21E	3.69''	0.97	H	P
9E vs. 33E	7.33'''	2.77	H	P
21E vs. 33E	5.76'''	1.81	H	P
12E vs. 24E		5.30***	H	P
12E vs. 36E	2.43	0.72	H	P
36E vs. 24E		3.95**	H	P

' $F1_{(4,76)} = 2.96$ at P = .025. * $F2_{(4,155)} = 2.88$ at P = .025.

'' $F1_{(4,76)} = 3.59$ at P = .01. ** $F2_{(4,155)} = 3.47$ at P = .01.

''' $F1_{(4,76)} = 5.18$ at P = .001. *** $F2_{(4,155)} = 4.94$ at P = .001.

EV.= expected vector and CV.= covariance matrix for the One Sample Test(').

H Heterogeneous variances ($P \leq .05$).

P Mean response vectors are parallel ($P \leq .05$).

Figure 4.12
Mean response vectors of 7, 11, and 13 day old test larvae to 1, 3, 5, or 7 days of conditioning done by a 12 day old larva.

A. CAT=1,12,(7,11,13)

B. CAT=3,12,(7,11,13)

C. CAT=5,12,(7,11,13)

D. CAT=7,12,(7,11,13)

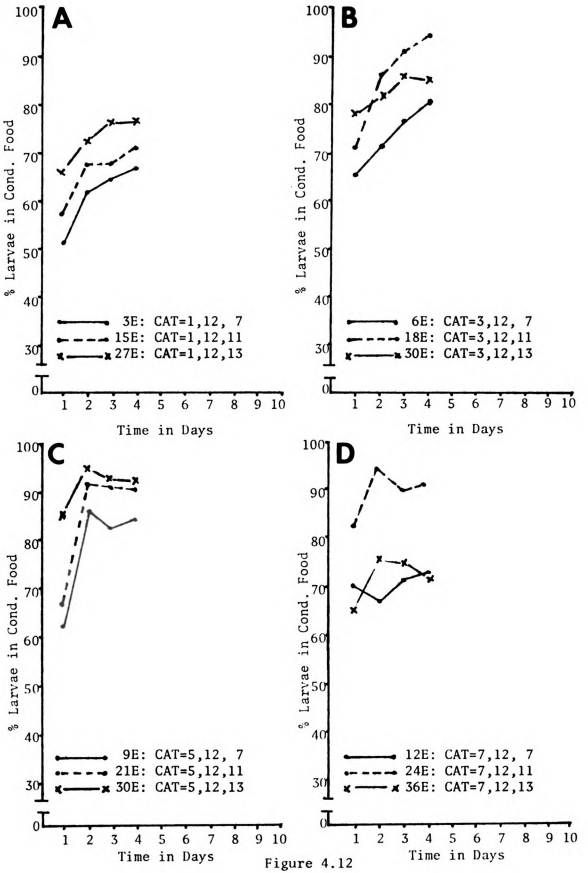

A
% Larvae in Cond. Food
Time in Days
3E: CAT=1,12, 7
15E: CAT=1,12,11
27E: CAT=1,12,13
B
% Larvae in Cond. Food
Time in Days
6E: CAT=3,12, 7
18E: CAT=3,12,11
30E: CAT=3,12,13
C
% Larvae in Cond. Food
Time in Days
9E: CAT=5,12, 7
21E: CAT=5,12,11
30E: CAT=5,12,13
D
% Larvae in Cond. Food
Time in Days
12E: CAT=7,12, 7
24E: CAT=7,12,11
36E: CAT=7,12,13

Figure 4.12

[comparison 25E versus 34E, Table 4.10, Figure 4.10D], and CAT=7,9 (7,13) [comparison 11E versus 35E, Table 4.11, Figure 4.11D].

However, examination of Figures 4.10, 4.11, and 4.12 reveals some apparent age differences in response which the Two-Sample analysis is not picking up. For example, in Figure 4.11A curves 2E and 26E appear very different but the Two-Sample procedure says they are the same. Again we have the problem of heterogeneity of variances. When all of these comparisons were rerun with the One-Sample approach (F1-values in Tables 4.10, 4.11, and 4.12) many more age differences become apparent, and not just at high levels of conditioning. Rather now the trend is a consistent one of age differences in response being at all levels of conditioning, which biologically makes more sense. Comparisons of Figures 4.10, 4.11, and 4.12 reveals that 7 day old larvae consistently respond at a lower level to the same degree of conditioning than 11 or 13 day old larvae and 11 day old larvae are variable, being intermediate between 7 and 13 day old larvae or not different from 13 day old larvae. Figure 4.12D shows the "atypical" groups (12E, 24E, and 36E) as earlier discussed.

If these differences are age-related they should disappear when comparing segments of the response vectors where ages are compatible. For example, comparing the first 4 days of the 13 day old response vector with the last 4 days of the 7 or 11 day old vectors, would result in comparing each test age group from the time the larvae are 13 days old until they are 16 days old. To get some indication if the responses in Tables 4.10, 4.11 and 4.12 are age-related these comparisons were only made for those groups shown to be different for

the first 4 days by the Two-Sample test. The results are shown in Table 4.13.

The results in Table 4.13 show that, except for comparison 22E versus 34E, the response vectors remain significantly different even for comparable segments of the vectors. It will also be noted that the two significant comparisons with the Two-Sample procedure exhibit non-parallel responses, which means that the larvae may now be exhibiting different behaviors, rather than different degrees of the same behavior. However, we must be very careful about interpreting these results because of the confounding variables, early experience in the colony dishes and length of time in the experimental situation. These will be discussed later.

Table 4.13

Results of Hotelling's One (') and Two (*) Sample Tests on comparable 4 day segments of the response vectors shown to exhibit age differences by a Two-Sample comparison in Tables 4.10, 4.11, and 4.12.

Comparisons EV. CV.	F_1	F_2	Box's Test	T^2 Test for Parallelism
10E vs. 34E		5.70**	H	
22E vs. 34E	20.09"	2.79	H	P
11E vs. 35E		5.13**	H	

'$F_{1(4,76)} = 2.96$ at P = .025.

"$F_{2(4,76)} = 5.18$ at P = .001.

H Heterogeneous variances ($P \leq .05$).

P Mean response vectors are parallel ($P \leq .05$).

*$F_{2(4,155)} = 2.88$ at P = .025.

**$F_{2(4,155)} = 3.47$ at P = .001.

Since the groups in Figure 4.2 were run at different times and with larvae from different populations the question arose as to whether behavior is constant over time and from group to group. As a test of this question, replications were periodically run and their mean response vectors compared to the original experimental groups. Nineteen such replications (Figure 4.13) were performed and the results are shown in Table 4.14. The replicates (R) are numbered as their corresponding experimental groups, the only differences being that the replicates were run at some time after the experimental groups and with different larval hatches. In every case, the F1- and F2-values show no difference, but in 13 of 19 comparisons the variances are heterogeneous, which means that behavior is fluctuating from population to population, although the response examined is constant.

IV. A Brief Analysis Relating to Initial Preference to Conditioned Food and the Amount of Larval Movement as Conditioning Increases

A. Does Larval Initial Preference to Conditioned Food Change as Degree of Conditioning Increases?

It can be seen from examination of Figures 4.4, 4.5, and 4.6 that most initial preferences, i.e., the first point of each response mean vector are probably not good predictors of maximum response to X degrees of conditioning. However, a comparison of initial preference to low versus high conditioning will give us some idea if differences do exist. The comparisons made deal with CAT=1,9(7,11,13) vs. CAT=7,9(7,11,13). The analysis is by means of a 2 x 2 contingency

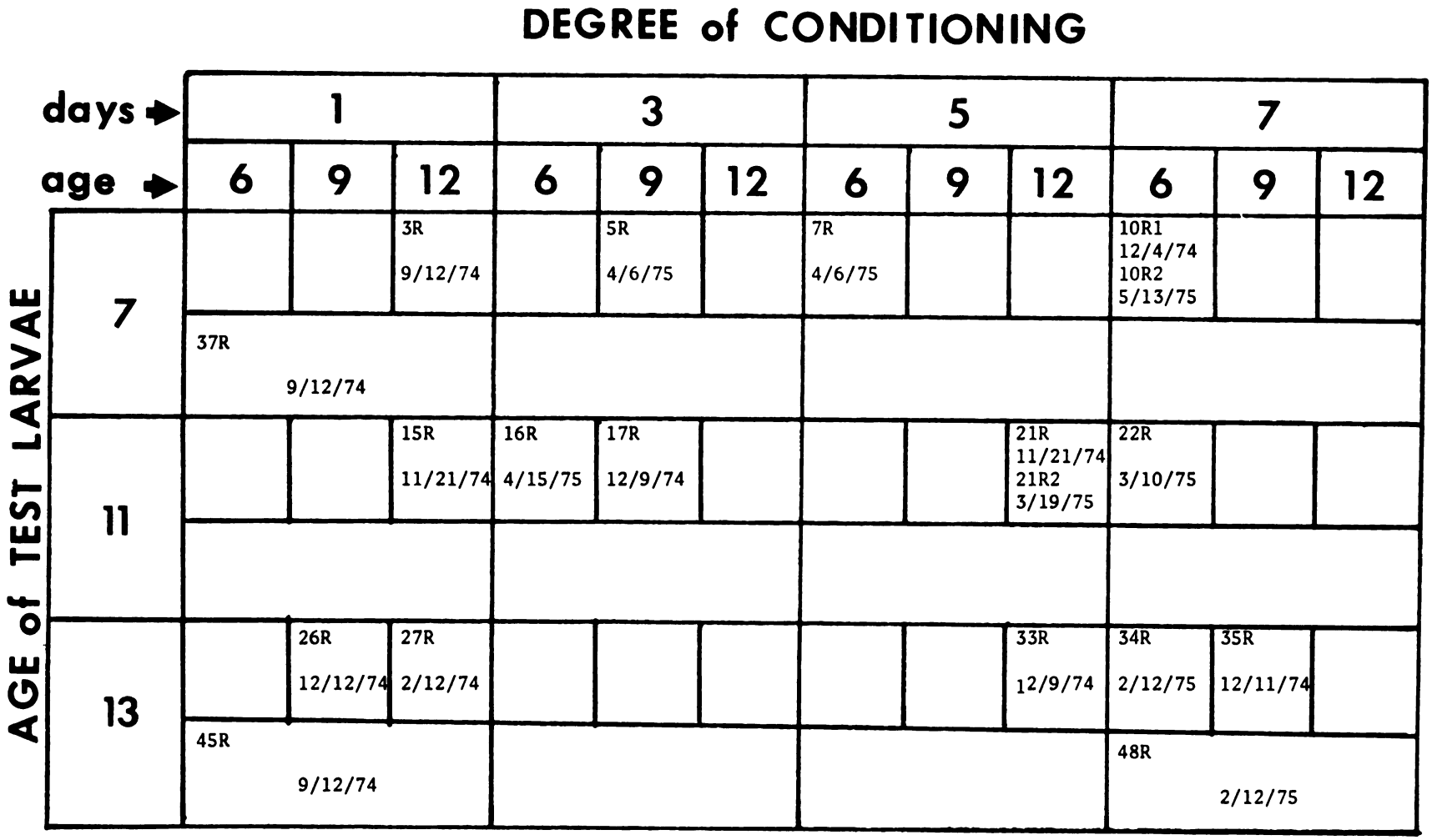


Figure 4.13
Replications of randomly selected groups from the experimental design in Figure 4.2.

Table 4.14

Hotelling's One(') and Two(*) Sample comparisons of randomly selected experimental groups from Figure 4.2 with their replications as a test of whether behavior is changing over time and from hatch to hatch.

Groups		Time in Days 1	2	3	4	5	6	7	8	9	10	F1	F2	D	Box's Test
3E vs. 3R	$\bar{X}_{3E}$	.512	.625	.650	.675	.700	.687	.737	.712	.737	.675			1.54*	
	$\bar{X}_{3R}$	.537	.550	.662	.662	.737	.725	.750	.725	.687	.525		1.93	1.39*	
	S^2_{Pooled}	.252	.244	.228	.224	.204	.216	.193	.205	.207	.237				
5E vs. 5R	$\bar{X}_{5E}$	.562	.700	.762	.750	.800	.837	.800	.812	.712	.612		0.17	1.39*	
	$\bar{X}_{5R}$	.575	.725	.762	.775	.812	.825	.775	.800	.675	.625			1.35*	
	S^2_{Pooled}	.248	.207	.183	.183	.158	.142	.169	.158	.215	.239				
7E vs. 7R	$\bar{X}_{7E}$	.625	.750	.787	.750	.812	.787	.762	.775	.775	.712		0.34	1.37*	
	$\bar{X}_{7R}$	.600	.737	.762	.775	.850	.787	.775	.750	.762	.687			1.70	
	S^2_{Pooled}	.240	.193	.176	.183	.142	.169	.180	.183	.180	.212				
10E vs. 10R1	$\bar{X}_{10E}$	.725	.800	.887	.837	.875	.912	.900	.875	.925	.737	1.81	1.01	1.65*	
	$\bar{X}_{10R1}$	.725	.825	.862	.887	.900	.887	.900	.925	.925	.850			1.34*	
	S^2_{10E}	.202	.162	.101	.138	.111	.081	.091	.111	.070	.196				H
	S^2_{10R1}	.202	.146	.120	.101	.091	.101	.091	.070	.070	.129				
	S^2_{Pooled}	.202	.154	.111	.119	.101	.091	.091	.091	.070	.163				

'$F1_{(10,70)} = 2.24$ at P = .025. *$F2_{(10,149)} = 2.15$ at P = .025. $D_L = 1.71$ and $D_U = 2.28$ at P = .05.

I D-value was inconclusive.

H Heterogeneous variances ($P \leq .05$)

Table 4.14 (cont'd.)

Groups		Time in Days 1	2	3	4	5	6	7	8	9	10	F1	F2	D	Box's Test
10E vs. 10R2	$\bar{X}_{10E}$	.725	.800	.887	.837	.875	.912	.900	.875	.925	.737	1.71	0.75	1.65*	
	$\bar{X}_{10R2}$	.712	.887	.875	.837	.912	.900	.900	.887	.887	.762			1.73I	
	S^2_{10E}	.202	.162	.101	.138	.111	.081	.091	.111	.070	.196				H
	S^2_{10R2}	.207	.101	.111	.138	.081	.091	.091	.101	.101	.177				
	S^2_{Pooled}	.205	.132	.106	.138	.096	.086	.091	.106	.086	.199				
10R1 vs. 10R2	$\bar{X}_{10R1}$	.725	.825	.862	.887	.900	.887	.900	.925	.925	.850	1.90	1.14	1.34*	
	$\bar{X}_{10R2}$	.712	.887	.875	.837	.912	.900	.900	.887	.887	.762			1.73I	
	S^2_{10R1}	.202	.146	.126	.101	.091	.101	.091	.070	.070	.129				H
	S^2_{10R2}	.207	.101	.111	.138	.081	.091	.091	.101	.101	.177				
	S^2_{Pooled}	.205	.124	.115	.119	.086	.096	.091	.086	.086	.166				
37C vs. 37R	$\bar{X}_{37C}$	.512	.475	.487	.562	.537	.550	.500	.512	.500	.475		0.81	1.33*	
	$\bar{X}_{37R}$	.487	.525	.525	.537	.525	.500	.512	.537	.525	.425			1.44*	
	S^2_{Pooled}	.253	.253	.253	.250	.252	.252	.253	.252	.253	.256				

'$F1_{(10,70)} = 2.24$ at P = .025. *$F2_{(10,149)} = 2.15$ at P = .025. $D_L = 1.71$ and $D_U = 2.28$ at P = .05.

ID-value was inconclusive.

HHeterogeneous variances ($P \leq .05$).

Table 4.14 (cont'd.)

Groups		Time in Days 1	2	3	4	5	6	F1	F2	D	Box's Test
15E vs. 15R	$\bar{X}_{15E}$	.575	.687	.687	.725	.775	.762	1.63	0.71	1.72I	
	$\bar{X}_{15R}$	.562	.737	.725	.775	.812	.775			1.92I	
	S^2_{15E}	.247	.218	.218	.202	.177	.183				
	S^2_{15R}	.249	.196	.202	.177	.154	.177				H
	S^2_{Pooled}	.248	.207	.190	.186	.165	.165				
16E vs. 16R	$\bar{X}_{16E}$	.725	.712	.687	.675	.687	.687		0.72	1.82I	
	$\bar{X}_{16R}$	.687	.737	.725	.700	.675	.675			2.09I	
	S^2_{Pooled}	.210	.202	.210	.217	.220	.220				
17E vs. 17R	$\bar{X}_{17E}$	.737	.850	.800	.850	.812	.800	0.43	0.15	1.85I	
	$\bar{X}_{17R}$	.762	.862	.825	.862	.850	.800			1.94I	
	S^2_{17E}	.196	.129	.162	.129	.154	.162				
	S^2_{17R}	.183	.120	.146	.120	.129	.162				H
	S^2_{Pooled}	.190	.125	.154	.125	.142	.162				
21E vs. 21R1	$\bar{X}_{21E}$	.675	.937	.925	.912	.875	.850	1.24	0.61	2.06I	
	$\bar{X}_{21R1}$	.725	.937	.925	.937	.937	.925			2.01I	
	S^2_{21E}	.222	.059	.070	.081	.111	.129				
	S^2_{21R1}	.202	.059	.070	.059	.059	.070				H
	S^2_{Pooled}	.212	.059	.070	.070	.085	.100				

'$F1_{(6,74)} = 2.59$ at P = .025. *$F2_{(6,153)} = 2.51$ at P = .025.

$D_L = 1.83$ and $D_U = 2.16$ at P = .05. I = D-value was inconclusive.

HHeterogeneous variances ($P \leq .05$).

Table 4.14 (cont'd.)

Groups		Time in Days 1	2	3	4	5	6	F1	F2	D	Box's Test
	$\bar{X}_{21E}$	.675	.937	.925	.912	.875	.850	1.68	0.33	2.06I	
21E	$\bar{X}_{21R2}$	.700	.950	.937	.975	.975	.925			2.13I	
vs.	S^2_{21E}	.222	.059	.070	.081	.111	.129				H
21R2	S^2_{21R2}	.213	.048	.059	.025	.025	.070				
	S^2_{Pooled}	.217	.054	.065	.053	.068	.100				
	$\bar{X}_{21R1}$	.725	.937	.925	.937	.937	.925	1.13	0.60	2.01I	
21R1	$\bar{X}_{21R2}$	.700	.950	.937	.975	.975	.925			2.13I	
vs.	S^2_{21R1}	.202	.059	.070	.059	.059	.070				H
21R2	S^2_{21R2}	.213	.048	.059	.025	.025	.070				
	S^2_{Pooled}	.207	.054	.065	.042	.042	.070				
	$\bar{X}_{22E}$	.637	.850	.887	.862	.875	.862	1.18	0.37	2.05I	
22E	$\bar{X}_{22R}$	.650	.812	.900	.862	.862	.875			1.67*	
vs.	S^2_{22E}	.234	.129	.101	.120	.111	.120				H
22R	S^2_{22R}	.230	.154	.091	.120	.120	.115				
	S^2_{Pooled}	.232	.142	.096	.120	.115	.115				

†$F1_{(6,74)} = 2.59$ at P = .025. *$F2_{(6,153)} = 2.51$ at P = .025.

$D_L = 1.83$ and $D_U = 2.16$ at P = .05. I = D-value was inconclusive.

HHeterogeneous variances ($P \leq .05$).

Table 4.14 (cont'd.)

Groups		Time in Days 1	2	3	4	F1	F2	D	Box's Test
26E vs. 26R	$\bar{X}_{26E}$	.662	.662	.650	.650		0.22	2.21	
	$\bar{X}_{26R}$	.662	.662	.675	.650			2.21	
	S^2_{Pooled}	.226	.226	.226	.230				
27E vs. 27R	$\bar{X}_{27E}$	.662	.737	.775	.775	1.89	0.94	2.31	
	$\bar{X}_{27R}$	.750	.775	.787	.837			2.31	
	S^2_{27E}	.226	.196	.177	.177				H
	S^2_{27R}	.196	.177	.169	.138				
	S^2_{Pooled}	.208	.186	.173	.157				
33E vs. 33R	$\bar{X}_{33E}$	.850	.950	.937	.925	0.20	0.09	2.20	
	$\bar{X}_{33R}$	.850	.950	.950	.925			2.52	
	S^2_{33E}	.129	.048	.059	.070				H
	S^2_{33R}	.129	.048	.048	.070				
	S^2_{Pooled}	.129	.048	.054	.070				
34E vs. 34R	$\bar{X}_{34E}$	.925	.975	.987	.975	2.25	0.57	2.33	
	$\bar{X}_{34R}$	.912	.962	.962	.975			2.22	
	S^2_{34E}	.070	.025	.012	.025				H
	S^2_{34R}	.081	.037	.037	.025				
	S^2_{Pooled}	.076	.031	.025	.025				

$F1_{(4,76)} = 2.96$ at P = .025. $^{*}F2_{(4,155)} = 2.88$ at P = .025.

$D_L = 1.89$ and $D_U = 2.10$ at P = .05. I = D-value was inconclusive.

^{H}Heterogeneous variances ($P \leq .05$).

Table 4.14 (Con't.)

Groups		Time in Days 1	2	3	4	F1	F2	D	Box's Test
	$\bar{X}_{35E}$	.875	.975	.987	.962	0.02	0.09	2.47	
35E	$\bar{X}_{35R}$	.850	.962	.987	.962			2.20	
vs.	S^2_{35E}	.111	.025	.012	.037				H
35R	S^2_{35R}	.129	.037	.012	.037				
	S^2_{Pooled}	.120	.031	.012	.037				
	$\bar{X}_{45C}$	.462	.525	.487	.525		0.55	2.32	
45C	$\bar{X}_{45R}$	.462	.487	.462	.462			2.01I	
vs. 45R	S^2_{Pooled}	.252	.253	.252	.252				
	$\bar{X}_{48C}$	.450	.437	.462	.475		0.87	1.96I	
48C	$\bar{X}_{48R}$	.475	.500	.500	.487			2.23	
vs. 48R	S^2_{Pooled}	.252	.251	.252	.253				

'$F1_{(4,76)} = 2.96$ at P = .025. *$F2_{(4,155)} = 2.88$ at P = .025.

$D_L = 1.89$ and $D_U = 2.10$ at P = .05. I = D-value was inconclusive.

HHeterogeneous variances ($P \leq .05$).

table with a Chi-square analysis and the results are shown in Table 4.15.

The results in Table 4.15 indicate that, at least initially, 7 and 11 day old larvae do not discriminate between low and high degrees of conditioning. However, when the total mean response vectors for 2E versus 11E and 14E versus 23E are compared (Tables 4.4 and 4.5) differences in response are detected. Therefore, initial preference is not a good predictor for long-term preference. The 13 day old test larvae, on the other hand, does discriminate in its initial response to low versus high conditioning. There would therefore appear to be an age difference in the ability to discriminate between varying degrees of conditioning. However, if the initial

Table 4.15

Chi-square analysis of initial larval preference to the extremes of conditioning within the three test ages.

Age of Test Larvae	Comparisons	Number in Cond. food	Number in Plain food	Calculated X^2-value
7	Low Cond.(2E)	39	41	3.0636
	vs.			
	High Cond.(11E)	50	30	
11	Low Cond.(14E)	51	29	2.9760
	vs.			
	High Cond.(23E)	61	19	
13	Low Cond.(26E)	53	27	7.4295**
	vs.			
	High Cond.(35E)	70	10	

*$X^2_{(1)}$ = 3.81 at P = .05.

**$X^2_{(1)}$ = 6.635 at P = .01.

response of 7 day old larvae to high conditioning versus low conditioning (50 versus 30) is compared, by means of a 2 x 2 contingency table with Chi-square analysis, with the initial response of a 13 day old larva to high versus low conditioning (70 versus 10) a X^2-value of 13.33 results. This is significant at the .005 level. Therefore, not only do 13 day old larvae show a higher discriminating between low versus high conditioning but their initial preference to high conditioning is greater than for that of a 7 day old larva.

B. Does Amount of Larval Movement Vary in Different Amounts of Conditioning?

Experiments up to this point have shown that larvae prefer conditioned food over non-conditioned food and that as conditioning increases the frequency of larvae found in conditioned food increases. This would indicate that larvae prefer to settle in conditioned food and that the number of movements from plain food to conditioned food should increase as conditioning increases. As a partial test of this hypothesis it was determined how many times larvae moved to conditioned food and vice versa, for each of the test ages in a low-conditioned situation (groups 2E, 14E, and 26E) and a highly-conditioned situation (groups 11E, 23E, and 35E). The results are shown in Table 4.16. To equalize number of days spent in the experimental situation, only counts for the first 4 days were used in this analysis.

The results in Table 4.16 show that, regardless of age, significantly more larvae move to the highly conditioned food than move away from it. At low conditioning, however, only 7 day old larvae show a significant difference. Examination of the observed frequencies

Table 4.16

Results of a chi-square goodness of fit analysis on the number of times larvae moved between conditioned and plain food lumps when conditioning is either low or high.

Conditioning	Age of Test Larvae	Group	Comparison	Observed Number	Expected Number	Calculated X^2-values
LOW	7	2E	Moved to Cond.	20	14.5	4.1724*
			Moved to Plain	9	14.5	
	11	14E	Moved to Cond.	20	15.5	2.62
			Moved to Plain	11	15.5	
	13	26E	Moved to Cond.	10	11	0.1818
			Moved to Plain	12	11	
HIGH	7	11E	Moved to Cond.	20	14.5	4.1724*
			Moved to Plain	9	14.5	
	11	23E	Moved to Cond.	15	8.5	4.1720*
			Moved to Plain	2	8.5	
	13	35E	Moved to Cond.	11	7	4.5714*
			Moved to Plain	3	7	

$X^2_{(1)} = 3.841$ at $P = 0.05$.

also shows that, regardless of the degree of conditioning, the number of movements of 7 or 11 day old larvae (younger larvae) are greater than for older 13 day old larvae. For example, in high conditioning, 7 day old larvae moved a total of 29 times in 4 days as opposed to only 14 times for 13 day old larvae. In a chi-square goodness of fit test this yields a X^2-value of 5.2324 which is significant at the 0.025 level. The number of movements by an 11 day old larvae however, is not different from either 7 or 13 day old movements. No differences exist between total movements in low conditioning for any of the three test ages.

Control for Food Limitation

Before making conclusions about the effects of homotypic conditioning on the distribution of isolate larvae, it is first necessary to demonstrate that food is not a limiting factor. To demonstrate this, an experiment was run in which developmental time on a maximally conditioned food lump was compared to developmental time on a plain food lump. A six-group experiment was set up with N=20 per group. In three groups each dish had a single food lump conditioned by a 9 day old larva for 7 days. After conditioning the 9 day old larvae were removed and isolate 7, 11, or 13 day old larvae reared until pupation. The other three groups were identical except the food was not conditioned. If developmental time (pupation time) does not differ among these six groups, then food was not a limiting factor in my experiments. The results of an analysis of variance on this data is shown in Table 4.17 and it can be seen that all pupation times were the same. The means and standard deviations for the 7, 11, and 13 day old

Table 4.17

Analysis of variance on developmental times of 7, 11, and 13 day old larvae reared on single food lumps which were either maximally conditioned or non-conditioned.

Source of Variation	d.f.	S.S.	M.S.	F ratio
Between Group	5	0.7416	0.1483	0.2372
Within Group	114	71.2500	0.625	
Total	119	71.9916		

$F_{(5,119)} = 2.2914$ at $P = .05$.

isolates reared on a single unconditioned food lump were 18.35 $\pm$ 0.55, 18.50 $\pm$ 0.68, and 18.55 $\pm$ 0.57, respectively. The means and standard deviations for the 7, 11, and 13 day old isolates reared on a single conditioned food lump were 18.55 $\pm$ 0.78, 18.60 $\pm$ 0.56, and 18.50 $\pm$ 0.57, respectively.

V. Can Response to Conditioning Be Overridden by Varying the Food Source?

Park (1948) stated that there is probably some kind of interaction between conditioning and food source involved in the response of Tribolium to conditioned food. With Galleria larvae, I was interested in finding out if larval initial preferences distinguish between conditioned food sources which are either poor in nutrients or rich in nutrients. In order to make such a test, a diet (deficient diet) was needed on which development is delayed. The following diet was used: Pablum = 43.16g, yeast = 0g, honey = 7.5g, glycerin = 31.16g, water = 18.16g, and beeswax = 0g, whereas the normal diet consists of pablum = 32.98g, yeast = 9.7g, honey = 24.25g, glycerin = 21.34g,

water = 8.73g, and beeswax = 3g. Sixteen 11 day old larvae were reared on single 4 gram lumps of deficient diet and sixteen on single lumps of normal diet (the normal diet being that used in all previous experiments). The ANOVA in Table 4.18 demonstrates that development was slower on the deficient diet, it being 26.12 $\pm$ 0.52 days on deficient and 18.37 $\pm$ 0.3 days on normal food.

Table 4.18

Analysis of variance on 11 day old larvae reared on normal and deficient diets.

Source of Variance	d.f.	S.S.	M.S.	F ratio
Between Group	1	480.5	480.5	168.5964*
Within Group	30	85.5	2.85	
Total	31	560.00		

*$F_{(1,31)}$ = 13.2222 at P = 0.001.

To determine if larvae exhibit a preference for normal over deficient food, 16 dishes were set up, each with one normal and one deficient 4-gram food lump on which no conditioning was allowed. One 11 day old larva was tested for preference on each dish. Data was collected for initial preference (the preference 5 minutes after being placed in the experimental situation) and preference 24 hours later. The results were that all 16 larvae preferred the normal food lump at both data points.

I next set up an experiment consisting of two groups of 16 dishes each. In one group each dish had two normal food lumps one of

which was conditioned. In the other group, each dish had one normal food lump and one deficient conditioned lump. Tests were made with one 11 day old larva per dish and data collected for initial and 24 hour preferences. The data being analyzed is the number of times larvae that initially selected the deficient conditioned lump or the normal conditioned lump switched (flipped) to the normal plain lump in 24 hours. The results are shown in Table 4.19, where DC = deficient-conditioned lump and NC = normal-conditioned lump.

Table 4.19

A 2 x 2 contingency table analyzed by chi-square of larval responses to normal-conditioned and deficient-conditioned food.

	DC	NC	
Flip	8	0	8
No Flip	2	16	18
	10	16	26

Calculated X^2 = 18.5287*

*X^2 = 7.879 at P = 0.005.

There is therefore, an interaction between food source and conditioning. Initial preference is to conditioning but food preferences will over-ride the initial preference in the deficient food situation. It should be noted, however, that there is also a difference in initial preference for deficient-conditioned versus normal-conditioned food. Only 10 larvae initially selected the deficient conditioned lump and then 8 of the 10 flipped, whereas all 16 larvae selected the normal-conditioned lump with no flips.

VI. Do Adult Galleria Exhibit Preferences to Conditioning Done by Larvae?

Adult Galleria (metamorphosed) do not eat and are not normally found in the larval food source, and conditioning would probably have very little attraction for them from a feeding point of view. Paddock (1913) and Oertel (1962) both indicate that adult females exhibit preferences for oviposition sites and Oertel (1962) further demonstrated that adults are attracted to bee hives which have been larval conditioned. I therefore decided to ask if female adults prefer to lay their eggs in conditioned food.

Two groups of 20 dishes were set up. In one group each dish had 2, 4-gram normal food lumps, one of which was conditioned by a 9 day old larva for 3 days (low conditioned). In the other group one of the lumps was conditioned by a 9 day old larva for 7 days. The conditioning larvae were then removed and one female adult was placed in each dish. The females were taken from the breeding tank. After 5 days the eggs laid on each food lump were counted and each group analyzed by a chi-square goodness of fit test as shown in Table 4.20.

Female adults exhibit no distinction between low-conditioned food and non-conditioned food for egg laying. However, they prefer non-conditioned food to highly conditioned food for egg-laying.

Discussion

Studies with invertebrates indicate that biological conditioning affects spatial distribution. These studies, however, deal with whole populations of organisms and are confounded by animal-animal interactions, sexual differences, and various environmental parameters.

Table 4.20

Chi-square goodness of fit test on female adult preference for egg-laying in low and high larval conditioned food.

Comparison	Observed Number of eggs	Expected Number of eggs	Calculated X^2-values
Low Cond. Food	5705	5785.5	1.3980
vs.			
Plain Food	5832	5768.5	
High Cond. Food	3742	5019	649.8222*
vs.			
Plain Food	6296	5019	

*$X^2_{(1)}$ = 7.879 at P = 0.005.

No systematic attempt has been made to elucidate the effect of conditioning on individual behavior. Park's work (1934, 1935), for example, is confounded by the presence of every developmental stage in his culture dishes, and Naylor's (1959) studies were unable to definitively separate conditioning effects from sexual or density factors. Wellington (1957) developed a bioassay for individual behavioral response of the tent caterpillar to light, but not to conditioning, and some of the responses of his Type II (less active) larvae could be interpreted in terms of lack of proper stimuli from homotypic conditioning. Surtees (1963a, 1964e) completely ignored the effects that biological conditioning may have been having on populations of Tribolium and various grain beetles, and it is not clear from Long's (1955) data if aggregation formation and feeding behavior

in butterfly larvae are a function of biological conditioning, animal-animal interactions, or both.

The hypothesis for my studies in this paper has been that the spatial distribution of isolate Galleria larvae is a function of larval conditioning and that preference for conditioned medium is a function of the degree of conditioning and age of responding larvae.

Larval Responses to Increasing Degrees of Conditioning

The between-group comparisons of larval responses to conditioning showed that response increases as days of conditioning increase, as age of conditioning larvae increase, or as a function of the two. Older larvae may be conditioning the medium more than younger larvae or they may be differentially conditioning the medium. In every case examined, individual responses to conditioning are either random or attraction. Avoidance was never seen, but a response plateau was indicated. There may be a threshold effect in which conditioning beyond a certain level will not elicit a greater response. This requires further investigation particularly in reference to whether conditioning beyond that used in my experiments may elicit avoidance reactions from the test larvae. Such experiments, however, would need to carefully control for avoidance to food limitation. It may be that repulsion to conditioning is not an appropriate behavior unless the conditioned medium is also food limited. Park (1948) noted that Tribolium was repelled by excessive conditioning, but his system was food limited in that the medium used was two year old colony food.

Age Differences in Response to Conditioning and Possible Mechanisms

The data on age differences in response to the same degree of conditioning are not as clear. Significant age differences in response were evident only at the highest degrees of conditioning when the Two-Sample comparison was utilized and at all degrees of conditioning when the One-Sample test was used. There is also every indication (Table 4.13) that these differences do not disappear by comparing identical segments of the mean response vectors.

Two-Sample comparisons in Table 4.13 show that not only do age differences not disappear by comparing equal segments of the response curves, but that non-parallelism tends to result as well, indicating the presence of interactions other than age or that behavior is qualitatively different. The age analyses may be confounded by differences in larval size, activity, sensory capabilities, and early experiences, both in the colony and the experimental apparatus.

Size of Responding Larvae

The size of different aged larvae may have been a factor in their ability to negotiate the distance between food lumps. As shown in Figure 4.1, the size difference between a 7 and a 13 day old larva is great. The distance between food lumps was 35 mm across the center of the dish. Therefore, 7 day old larvae had a greater relative distance to travel from one food lump to the other than did 13 or 11 day old larvae. If, for example, a 13 day old larva initially chose the plain food lump, it could extend part of its body out of that lump and almost encounter the conditioned lump. A 7 day old larva would

have to completely leave the plain food and travel a relatively large distance before encountering the conditioned lump.

Activity Differences

Galleria larvae may exhibit activity differences dependent upon age. Activity is being defined as amount of movement between food lumps, not within food lumps. Wellington (1957) showed that tent caterpillars are of several types as related to activity levels, some being active and others being sluggish and less investigatory. However, these types are genetically determined not age dependent, although Wellington's data is not very convincing. Long (1955), on the other hand, demonstrated that butterfly larvae exhibit different activity rhythms as development progresses. The older larvae became more active and tended to exhibit many investigatory excursions, and this developmental trend may be common to Lepidoptera. If 7 day old Galleria are initially less active and if response to conditioning is tactual rather than olfactory, they would tend to randomly select a food lump and remain there for a time. As they age, however, their activity pattern changes to investigatory and they move out to explore the surroundings. Assuming conditioning to be a preferred stimulus, when they encounter the conditioned food they stop moving and settle. This behavior would account for the low initial preferences of 7 day old test larvae and the gradual initial rise in their response vectors as they find the conditioned food. The more active 13 day old larvae investigate their surroundings immediately. Upon encountering conditioned food their preferred stimulus lessens their activity and they settle. This behavior is reflected in the higher initial preferences

shown by 13 day old larvae to high degrees of conditioning. Definitive experimental support for this hypothesis is lacking, but observational data and the mobility data in section IVA tend to support it. The mobility data shows more total movements between food lumps by 7 day old larvae than for 13 day old larvae, which indicates that 13 day old larvae find and settle in the conditioned food early, whereas 7 day old larvae do more exploratory movements.

However, the crucial point is not total activity of the different ages, but when the activity is taking place. If, for example, we re-examine Table 4.16, we find that 7 day old larvae moved a total of 29 times to only 14 times for 13 day old larva. This indicates that 7 day old larvae are more active than 13 day old larvae. However, the data sheets reveal that 7 day old larvae move a total of 6 times in the first two days of the experiment and 13 day old larvae moved a total of 7 times. This means that only 20.68% of the 7 day old movements occurred in the first two days to 50% for 13 day old larvae. Therefore, 7 day old larvae are, at least initially, relatively immobile and are not exploring their environment and finding the conditioned lump as soon as are older more active larvae. Even though the total mobility data shows 7 day old larvae to be more active than 13 day old larvae, this activity is occurring 3 or 4 days into the experiment at a time when the initially 7 day old larvae are older and apparently their activity has increased.

If this hypothesis is true, we would expect the response vectors for all degrees of conditioning to eventually reach the same level because all larvae will eventually find the conditioned food.

This does not happen. When a larva initially selects a plain food lump, it begins conditioning it. When investigatory movements set in, the larva may sample the initially conditioned lump but find that it is not as highly conditioned as the lump it just left and therefore return to its original choice. For example, the initial response of a 7 day old larva to conditioning done by a 9 day old larva for one day is low; that is, many 7 day old larvae initially chose the plain lump. Because their activity is low at this time, they remain in the plain lump and begin conditioning it. When they are older and their activity increases they begin to investigate their surroundings and encounter the initially conditioned lump. However, the conditioning they did for 3 or 4 days is greater than that done by the 9 day old larva for 1 day and they return to the "preferred" stimulus and settle. If the initially conditioned lump had more conditioning than the lump they left they will settle in it. Thus, the mean response vectors for low conditioning remain at a lower level than for high conditioning. These behaviors would necessitate some ability on the part of the larvae to discriminate between different levels of conditioning. Although there are indications of this ability in my studies it needs further testing.

Sensory Differences Between Ages

There may be sensory differences between ages, in which 13 day old larvae have better discriminatory powers for conditioning than do 7 day old larvae. The initial preference analysis in section IVA shows that, at least initially, 7 and 11 day old larvae do not distinguish between high and low conditioning, whereas 13 day old larvae do.

Whatever the mechanism(s), response to conditioning is more highly developed in older larvae. The analysis shows that there is a difference in initial preference of 7 or 13 day old larvae to highly conditioned food which tends to support a hypothesis relating to differential sensory ability. However, this initial preference data may also be interpreted in terms of activity differences. The significant initial preference of 13 day old larvae for conditioned food over plain food may be a reflection of their greater activity and early exploration of the experimental situation, whereas non-significance in 7 day old initial preferences for conditioned over plain food could be a reflection of their relative inactivity once any food lump is encountered.

Early Experience Factors

Early experience factors may also play a role in the age differences seen in my experiments. Seven day old test larvae are taken from the 7 day old colony dish which is less highly conditioned than the 11 or 13 day old colony dishes. Therefore, the older the test larva, the more early experience it has had with conditioning and with higher degrees of conditioning. This may account for a 13 day old larva's response to a high degree of conditioning being greater than a 7 day old's, and would be extremely difficult to control. One approach would be to raise larvae, for testing, as isolates and change their food every day, but this would not control for self-conditioning. Even if the food is changed daily a 13 day old larva would be experiencing its own conditioning which is greater than a 7 day old larva's.

Another approach would be to raise all larvae in mass cultures and base larval age for experimentation on larval size.

Finally, there may be an interaction between age and time spent in the experimental situation, 7 day old larvae spending more time in the dishes than 13 day old larvae. This would be difficult to control and still be able to follow total response mean vectors for each group. However, time spent in the experimental dishes is probably not a factor causing the differences in age. The analysis for age differences was initially with the first four days of the experimental period, regardless of age, and the differences were demonstrated.

Response to Biological Conditioning as a Bioassay of Behavior

The response of larvae to various degrees of homotypic conditioning is a good bioassay of individual behavior. The behavioral responses to graded series of conditioning has been shown to be constant for any given age and degree of conditioning. The replications of randomly selected groups in Figure 4.13 and Table 4.14 demonstrate that the response mean vectors do not change over time or from population to population of larvae, i.e., from hatch to hatch. However, these comparisons show that the variances between populations of larvae are heterogeneous. The behavioral response to conditioning may have become evolutionarily fixed with allowances for variability in how the response is achieved. That is, there are various strategies by which the same response is achieved.

If the response itself is of major interest, as it was in my studies, and not the variability in response from population to population, then the heterogeneous variances indicate the desirability of selectively breeding a "standard" Galleria stock for future experimentation. Response to conditioning could be used as a bioassay of the behavior being selected for.

As repeatedly mentioned and demonstrated throughout these studies the variances are heterogeneous. This has profound consequences on the power of the test to discriminate between a true and a false null hypothesis. Coupled with the positive serial correlation in my data two responses would have to be very different before being detected with the Two-Sample test, even with such large sample sizes. The power of the test was noticeably increased when the One-Sample test was employed, mainly because this procedure utilized the lower covariance matrix as the "best estimate" of the variances. Utilizing both procedures has enabled some insight into the behavior or Galleria larvae in responding to biological conditioning.

Aside from being heterogeneous, the variance vectors in Table 4.3 show a trend of decreasing magnitude as degree of conditioning increases. In other words, as conditioning increases the behavior of the larva tends to become more fixed. They select the conditioned food lump and show very little variation in response. If, however, conditioning is low the response is variable, with a good deal of movement between the conditioned and plain food. One might think of response in a low-conditioned situation as an equilibrium state in which there is a great deal of oscillation between food lumps, but

about a predictable level. As conditioning increases, however, these oscillations decrease in number as more and more larvae select the conditioned food lump and remain there. This trend in decreasing variance is also seen in the response of the 3 test ages to the same degree of conditioning. As larvae get older they tend to select and stay in conditioned food. This is probably related to the mobility differences discussed earlier.

There are any number of sources for variation in such experiments. Some of it may be due to environmental variability. Although light, temperature, and humidity were closely controlled there may have been undetected differences from experiment to experiment. However, these differences were not great enough to affect the results of larval selection as indicated by the replication data in Table 4.14.

Genetic variability may be the main contributor to the heterogeneous variances. Many experiments were run with different populations of larvae. Although controls and replications indicate this did not affect the behavior being looked at, the genetics may have been changing enough to account for the variability. This is impossible to determine at this time since the genetics of the larvae was not closely regulated or monitored.

An interesting fact from all my tables, however, is that even though the variance of any two groups is heterogeneous, the response curves are parallel. Parallelism indicates that, although at different levels, the response curves were achieved by similar behaviors. The heterogeneity may be a result of the degree to which each behavior is exhibited under varying degrees of conditioning. In selecting low or

high conditioned food, a larva utilizes its preference behavior for conditioning but this behavior increases and is less fluctuating as conditioning increases.

Possible Functions of Biological Conditioning

My studies have demonstrated that the spatial location of Galleria larvae may be largely determined by their homotypic conditioning. Individual larvae are attracted to conditioning and this attraction is a function of both the degree of conditioning and age of the larvae. These studies will be pursued further in the next chapter in relation to animal-animal interactions and resulting effects on individual preferences to conditioning.

Aside from its function in spatial distribution, biological conditioning may be important for other reasons. Some possibilities are that conditioning (1) serves as protection for the larvae, (2) provides a preferred consistency of the medium, (3) facilitates mobility, (4) signals the presence of other larvae, and (5) is related to feeding behavior.

Protection

Paddock (1913) hypothesized that spinning behavior in Galleria larvae serves as a scaffolding to prevent the hive combs from collapsing as the larvae tunnel throughout the hive and literally decimate it. Although this is an interesting hypothesis, there is no support for it.

Paddock (1913) also postulated that silk tunnels protect the larvae from the bees. The only support for this hypothesis is circumstantial. Free (1961) noted that bees sting dark colors, rough

surfaces, and rapidly moving objects. Galleria larvae, particularly older larvae, tend to be darkly colored and move in rapid, jerky movements. In their whitish, smooth, silken tunnels, however, their color and movements may go undetected. However, the interior of a bee hive is dark and vision on the part of the bees may not be utilized in detecting Galleria.

A possible test for both these hypotheses would be to artificially select for a population of Galleria that do not spin these tunnels. Comparisons could then be made to see if any selective advantage accrues to the spinning population.

Biological Conditioning as a Preferred Consistency

Larvae may prefer the consistency or the "feel" of biologically conditioned medium over non-conditioned medium. Although not a very tenable hypothesis, it does have minor support. Beck (1960) found that larvae preferred certain medium over others. Since he could not find any adaptive advantage to such a preference, he concluded that larvae like the consistency. Other workers, however, found a better explanation. Young (1961) found that the preferred medium enhanced larval growth, and Chase (1921) earlier noted that larvae raised on poor diets transform into smaller and less viable adults. However, consistency may well be a factor in their preferences if, for example, medium is easier to tunnel through.

Mobility

Mobility may be easier or quicker in conditioned than in non-conditioned food. This may be related to the silk itself or to the

fact that the tunnels provide ready access through the food. Stanley (1949), for example, demonstrated with Tribolium that tunnel-making in the food enhances mobility and that the beetles preferred using existing tunnels rather than constructing new ones.

A Signal for the Presence of Other Larvae

Biological conditioning may indicate the presence of other larvae. If individual larvae are responding to conditioning because it signals the presence of other larvae it would first be necessary to demonstrate that a larva is at least willing to tolerate the presence of other larvae. If it is not, then one would predict an avoidance to conditioning rather than an attraction. A partial test of this hypothesis is provided in Chapter V.

Biological Conditioning and Feeding Behavior

A final, and more probable, possibility is that preference for biological conditioning is related to feeding behavior. Brindley (1910) and Edwards (1910) demonstrated a relationship between silk threads spun by the processionary caterpillars and feeding excursions. Wellington (1957) showed that even relatively inactive larvae will follow silk threads to a food source, and Rathcke and Poole (1975) found that butterfly larvae (Mechantis isthmis) cooperate to spin a silk scaffolding over their food supply, enabling exploitation of an otherwise unavailable food source. Rathcke and Poole further postulated that such a massive silk gallery is energetically feasible only if several larvae pool their resources and share the benefits. Most

larvae spin some silk even if only during pupation and the evolution of a feeding web would entail only an elaboration of an already present ability. However, such behavior would require cooperation and possibly some form of social behavior. Long (1955) also demonstrated a relationship between biological conditioning and feeding behavior in larvae of the large white butterfly. Newly hatched larvae initially locate a food supply by following silk trails. Larvae also feed in masses, constantly spinning and enlarging a silk mat around the food supply.

Biological conditioning may signal an exploitable food supply to Galleria larvae. The higher the degree of conditioning means the better the food source, and so the greater the response to it, since the food supply would have to be nutritious enough for a larva to spend enough time on it to highly condition it. However, very highly conditioned food might also indicate a food depleted situation which might not affect larval initial preference for the conditioning. Haydak (1936), for example, showed that Galleria larvae reared on deficient diets produce less silk than those reared on normal diets. In my experiments with deficient diets, I had to use more larvae to condition a deficient food lump to the same degree as one larva's conditioning on normal food, as measured by response of a test larva. These experiments also show that, although initial preference is to conditioning, if the conditioned food source is deficient the larvae quickly move to a better non-conditioned source.

Biological Conditioning and Population Density

Biological conditioning may function as an index of larval population density. If this is true, then at some high degree of conditioning, larvae will avoid the conditioned situation. This was not observed in my experiments, probably because conditioning was not high enough, or food not limited as a result of the conditioning. However, a non-preference for highly conditioned food was seen on the part of adults to conditioning.

In 1959, Naylor stated that gravid female Tribolium tend to select, for egg laying, niche units of low adult and larval occupancy or avoid those of high occupancy. Chiang and Hodson (1950) had earlier demonstrated that larval Drosophila melanogaster inhibit oviposition of the adults and that oviposition is density-dependent on the larvae. My studies with egg laying preferences of female Galleria show that they avoid highly larval conditioned food for ovipositing, but exhibit a random response if food is only slightly conditioned. The biological conditioning may be functioning as an index of population density, and the survival value of the adult females' behavioral response is obvious. By laying her eggs in low or non-conditioned niches she ensures that hatching larvae will not be in extreme competition for the available resources which enhances their chances of survival to breeding age.

CHAPTER V

THE RELATIONSHIP BETWEEN BIOLOGICAL CONDITIONING AND ANIMAL-ANIMAL INTERACTION IN THE SPATIAL DISTRIBUTION OF GALLERIA MELLONELLA (L.) LARVAE

Introduction

In Chapter IV it was demonstrated that isolate Galleria larvae exhibit a preference for biologically conditioned food, that this preference increases as the degree of conditioning increases, and that possibly an age variable enters into the selection of conditioning. However, biological conditioning is a function of the organisms themselves and, as such, may be closely linked with interactions between other conspecifics. It is the purpose of this paper to look at how animal-animal interactions affect the preferences for conditioning established in Chapter IV.

Animal-animal interactions are known to affect the spatial distribution of organisms. Surtees (1963a, b, and c; 1964a, b, c, e and f) found in grain weevils that as density increased, the number of weevils appearing on the surface of the medium increased, but he failed to account for the extra conditioning as a result of increased density. A similar oversight was made by Legay and Chase (1964) who hypothesized that dispersal in Tribolium is dependent on exploration and population pressure due to increasing density. They attempted to explain their results purely on the basis of animal-animal interactions.

For example, they found the mean duration of stay of individual beetles in a partial enclosure to increase with increasing density. They interpret this as a result of accommodation to tactile stimuli among the beetles which had not discovered the way out of the enclosure. However, as density rises so does conditioning, and the "accommodation" to tactile stimuli may have been an increasing preference for increasing degrees of conditioning.

Naylor (1959 and 1965) found that female Tribolium confusum usually exhibit a uniform distribution but will form very loose aggregations in conditioning. Males on the other hand, aggregate in conditioning and with other males. His general conclusion was that spatial distribution in Tribolium is dependent upon density, but if sex and conditioning are added as variables the spatial patterns become altered. In 1963, Ghent contrasted the behavioral responses of Tribolium confusum and T. castaneum in mixed populations and demonstrated that the distributions of these populations depend upon animal-animal interactions, response to homotypic and heterotypic conditioning, and differences in behavior between the sexes.

Much of the work with Tribolium and other beetle populations is somewhat confounded by density and the effects of sexual behavior on response to biological conditioning. The effects of biological conditioning and animal-animal interactions may be more clearly seen in larval populations free from adult-young and sexual interactions. Galleria (larva) is such a model but has not yet been worked out. Edwards (1910) showed that larvae of the processionary caterpillar stay in feeding excursion lines as a result of silk strands and

head-to-tail larval contact, the latter being of predominant importance.

Several larval forms of butterflies exhibit a relationship between biological conditioning and animal-animal interactions (Long 1955). Silk threads are used to find food but larval-larval interactions serve to integrate the cyclic feeding behavior. Long also discovered that varying activity rhythms of different aged larvae affect the ability of individuals to join pre-established feeding groups. Similar kinds of behavior are noted by Wellington (1957 and 1960) with tent caterpillars. Different larval types can be identified on the basis of activity. Type I larvae are capable of independent, oriented movements, whereas Type II larvae need a silk trail or animal-animal contact for directed movements. Movements are even more directed if both stimuli are present.

Although demonstrated that biological conditioning and animal-animal interactions affect spatial patterns, the two variables are often confounded at the population level since there is little if any control over the amount of conditioning populations are doing. There have been only minor attempts at elucidating the effects of these variables on individual behavior. Knowing the relationship between individual preference for conditioning and degrees of conditioning (Chapter IV), it is the purpose of this paper to investigate whether these individual preferences are altered by the presence of another conspecific in the larvae of Galleria mellonella. The general hypothesis being tested is that individual preference for conditioning is unaffected by the presence of a conspecific and the resulting response

is a function of whether the conspecific is a resident in the conditioned food or introduced with the test larva.

Materials and Methods

Husbandry

All experimental larvae were reared as described in Chapter II and drawn from colony dishes maintained in darkness in incubators at 32 ± 1°C and 75% R.H. The experimental larvae used in these experiments are pictured in Figure 4.1 and their weights given in Table 4.1.

Apparatus

The experimental apparatus consisted of 100 x 25 mm plastic petri dishes with two 4 ± 0.05 gram food lumps in each dish. Within any one dish the food lumps were placed on opposite sides of the dish so that test larvae could be introduced between them and allowed to make a choice.

Experimental Procedures

The experimental procedures for all experiments in this paper are the same as described for the experiments in Chapter IV, with respect to how food is prepared and put in the experimental dishes, how conditioning is done and how test larvae are introduced and data collected. The only difference between the two procedures is that in these experiments two test larvae are added to each dish rather than one. These test larvae are of varying ages depending on the test situation. Both larvae were placed at the center of the dish and allowed to choose a food lump. Data was then collected on the

distribution of both larvae over time, although, in some situations, only the response of one of the larvae was monitored.

There are several different experimental designs in this paper and each will be described at the proper time. However, there are some nomenclature differences from Chapter IV. Only two different degrees of conditioning are utilized in these experiments, namely CAT=1,12,(7,11,13) and CAT=7,6,(7,11,13) which will be referred to as LOW and HIGH conditioning respectively, where C = days of conditioning, A = age of conditioning larvae, and T = age of test larvae.

Each experimental group has been assigned an identification code for ease in referring to the various comparisons I will be making. For example, codes such as 50P(1-3)7+7 or 64PH(1-5)7+13 will be frequently encountered. The first two numbers (50 or 64) are identification numbers for each experimental group and the rest of the nomenclature conveys the experiment that was performed. The P stands for "pairs" of larvae, meaning that two test larvae were used in the experiment, whereas an I stands for "isolate" in experiments when a single test larva was utilized (i.e., 50P(1-3)7+7 or 59I(1)7).

The letter following the P or the I will be either an L or an H referring to "low" and "high" degrees of conditioning. If neither an H or I appears in the identification number this means that when the experimental group was tested, at least initially, neither of the food lumps had been previously conditioned (50P(1-3)7+7). The two numbers on the end of the identification number (7+7 or 7+13) indicate the ages of the test larvae at the start of the experiment.

The numbers in parentheses refer to how the data for each experimental group is being analyzed. When two larvae are used in the experiments there are five possible ways in which to analyze the data:

1. total number of organisms on the conditioned food lump, regardless of whether paired or single;
2. total number of pairs on the conditioned food lump only;
3. total number of pairs regardless of the food lump on which they occur;
4. the number of older larvae of the pair of test larvae on the conditioned food lump, i.e., response of 13 day old larva when a 7 day old larva is also present;
5. the number of younger larvae of the pair of test larvae on the conditioned food lump, i.e., response of 7 day old larvae when a 13 day old larva is also present.

Larvae used in these experiments were not marked for identification purposes. Because I used such large sample sizes, marking them would have been prohibitive, and I would have had to mark them several times during the course of the experiment since they shed their cuticles several times throughout development. This would have necessitated disturbing the experimental procedures to remark them. Therefore, when two test larvae are the same age (i.e., 7+7, 11+11, or 13+13), individuals cannot be identified. Because of the size distribution of larvae, however, if two larvae are of different ages (i.e., 7+11, 7+13, or 11+13) individuals can easily be identified for analysis. Therefore, when both larvae are the same age, only the

first three of the above analyses are possible, whereas when the larvae are different ages all five of the analyses are possible.

In one experiment in this paper, neither food lump was pre-conditioned at the start of the experiment. In such a case the five possible analyses do not refer to the conditioned lump. However, one of the food lumps received an identification mark so that it could be distinguished from the other lump, making the five analyses possible as well. The following are a few examples of how to interpret the identification numbers in this paper:

50P(1-3)7+7: Experimental group #50, with a pair of test larvae with no initial conditioning, and in which the ages of the two larvae were 7+7. The possible analyses are (1-3) since both larvae are the same age.

60I(1)13 : Experimental group #60 with an isolate test larva of 13 days of age. When an isolate is used only the first analysis is ever utilized (1).

57PL(4)7+13: Experimental group #57 with a pair of test larvae tested with Low conditioning. The larvae are 7 and 13 days old and the analysis is on the 13 day old larva in conditioned food (4).

64PH(5)7+13: Experimental group #64 with a pair of test larvae that are 7 and 13 days old and High conditioning was employed. Analysis is on the response of the 7 day old larva to conditioned food (5).

68PH(5)7+<u>13</u>: Experimental group #68 with a pair of test larvae that are 7 and <u>13</u> days old and High conditioning was employed. The fact that the <u>13</u> is underlined indicates that this was a resident larva in the conditioned food and not introduced when the 7 day old test larva was. If neither age is underlined then both larvae were introduced together. Analysis is on the response of the 7 day old larva to conditioned food (5).

When two test larvae are of different ages, the length of the mean response vectors is dictated by the age of the older larva, the experiments being terminated when the older larva reaches its wandering phase. For example, if the two larvae are 7+13 days of age, only four experimental days will be analyzed. After 4 days the 13 day old larva enters the wandering phase. The 7 day old larva will not enter the wandering phase until 5 days after the 13 day old larva. However, I was interested only in animal-animal interactions during the "larval phase." Therefore, when the older larva enters the wandering phase, data analysis ends. There is one exception to this procedure which will be noted at the proper time.

Analysis

The dependent variable chosen for analysis within each experimental group is the mean response to conditioned food over time for pairs or either member of the pair of test larvae. When conditioned food was not employed, the dependent variable becomes the number of pairs, regardless of food lump, over time.

Each experimental group has 80 dishes and, in most cases, each dish has two test larvae. If both larvae are in the conditioned lump a score of 2 is recorded, if neither is in that lump a 0 is recorded. If only one larva is in the conditioned food a 1 is recorded, and in the case of different aged test larvae the 1 is identified as to which larva it represents. It was desirable to be able to compare two-larvae situations with isolate situations. To do this the sample sizes had to be adjusted. The following procedure was used.

If, for a two-larval test situation, it was desirable to analyze the number of pairs or the total number of larvae on a particular food lump in all 80 dishes over time, then the matrices would have entries of all two's or zeros for the paired situation and two's, one's, and zeros for total larvae on a particular food lump. These matrices were divided by the number of test larvae per dish, in this case 2, which reduces the entries to either one's, 0.5's, or zeros. These matrices are now comparable to matrices generated from only one test larva and the sample size is always 80 (the number of dishes) and not 160 and 80. If, on the other hand, the response of only one of the two test larvae is analyzed, the entries in its data matrix are already one's and zeros and need not be divided by 2.

Statistical analysis is by means of Hotelling's One- and Two-Sample procedures, as discussed in Chapters III and IV, although some Chi-square and ANOVA analyses are employed. All hypotheses are two-tailed and it was a priori determined to use an α-level of .025 because of heterogeneous variances, positive serial correlation, and multiple comparisons. These considerations were all discussed in Chapter IV.

Results

The following description of the results has been divided into three experiments, all of which relate to whether individual preference for biological conditioning is altered by the presence of a conspecific.

Experiment I: This experiment examines the question of whether, in the absence of initial conditioning, Galleria larvae aggregate and whether their distribution is a function of the age of the other member of the pair.

Experiment II: This experiment examines individual preferences for a conditioned food lump when a conspecific is also present and asks whether preference is a function of the conspecific, the age of the conspecific, or whether the conspecific is a resident in the conditioned food.

Experiment III: Experiment II will demonstrate that when 7+13 day old larvae are tested with high conditioning the 7 day old larva's response to conditioning is significantly lowered initially but increases with time. Experiment III deals with several test relating to whether this behavior is related to the behavior of the 13 day old larva, the conditioning, or an interaction between the two.

Experiment I: Do Galleria Larvae Group in the Absence of Initial Conditioning and Is their Distribution in Such a Situation a Function of the Presence of a Conspecific?

Before asking whether larval preferences for conditioning are affected by animal-animal interactions, it was decided to determine if larval-larval interactions affect their distribution when neither food lump is previously conditioned. The only valid measure to answer this question is the initial preference (5 minutes after the start of the experiment) of the larvae for being with a conspecific of varying ages or of remaining isolated. The larvae are constantly conditioning their environment so that very shortly after the experiment begins the food becomes conditioned and it would no longer be possible to determine if response is to the conditioning or the other larva. However, by looking at certain facets of the whole experiment, we can get some idea of which variable may be responsible for the resultant distributions.

The experimental design is shown in Figure 5.1. The six experimental groups are shown along with replications (R) of those

AGE OF TEST LARVAE

AGE OF TEST LARVAE	7	11	13
7	50P(1-3)7+7 9/11/74 50R(1-3)7+7 4/17/75	51P(1-5)7+11 9/11/74 51R(1-5)7+11 4/17/75	52P(1-5)7+13 9/11/74 52R(1-5)7+13 4/17/75
11		53P(1-3)11+11 9/11/74 53R(1-3)11+11 4/17/75	54P(1-5)11+13 9/11/74 54R(1-5)11+13 4/17/75
13			55P(1-3)13+13 9/11/74 55R(1-3)13+13 4/17/75

Figure 5.1

Experimental design for asking whether larvae will group (pair) in the absence of homotypic conditioning.

groups and the dates when each was run. All experimental groups were run at the same time and all replications were run together, but several months later. Each identification code indicates the larval combinations and the possible analysis and each group has a sample size of 80.

The question being asked is whether larvae will group (pair) in the absence of initial conditioning and when the age combinations of pairs is varied. The analyses utilized are in relation to pairing regardless of food lump, since both food lumps were "equal" at the start of the experiment.

Table 5.1 shows the results of comparing each experimental group with its replication by both the One- and Two-Sample procedures. The results indicate that, although variances are heterogeneous, pairing behavior in the absence of initial conditioning does not change from population to population of larvae.

Figure 5.2 (A+B) shows the mean response vectors for percent pairs of larvae on both food lumps when the food lumps were initially unconditioned. The only valid comparison for whether larvae prefer to pair in the absence of conditioning is for the first points on these curves. The results of this comparison may be found as Chi-square values in the first boxes of each comparison in Table 5.5. This table will be explained shortly, but for now the important point is that regardless of age combinations, pairs of larvae initially distribute themselves randomly. This indicates that, at least initially, the larvae are not reacting to each other. If such reactions were

Table 5.1

Results of Hotelling's One(') and Two(*) Sample comparisons of experimental groups(P) versus the replications(R) from Figure 5.1. Comparisons are made on the basis of total number of pairs, regardless of which food lump they were in.

Groups		Time in Days 1	2	3	4	5	6	7	8	9	10	F1	F2	D	Box's Test
	$\bar{X}_{50P}$	.575	.475	.475	.487	.575	.587	.637	.637	.675	.587	1.31	0.82	1.49*	
50P(3)7+7	$\bar{X}_{50R}$	.512	.525	.512	.575	.575	.612	.687	.612	.637	.650			1.50*	
vs.	S^2_{50P}	.247	.253	.253	.253	.247	.245	.234	.234	.222	.245				
50R(3)7+7	S^2_{50R}	.253	.253	.253	.253	.247	.247	.240	.218	.240	.234				H
	S^2_{Pooled}	.250	.253	.253	.250	.247	.243	.226	.237	.228	.238				
	$\bar{X}_{51P}$	.575	.550	.650	.687	.700	.850					1.23	0.83	1.85I	
51P(3)7+11	$\bar{X}_{51R}$	.562	.562	.650	.712	.800	.812							2.02I	
vs.	S^2_{51P}	.247	.251	.230	.218	.213	.129								
51R(3)7+11	S^2_{51R}	.249	.249	.230	.207	.162	.154								H
	S^2_{Pooled}	.248	.250	.230	.212	.187	.142								
	$\bar{X}_{53P}$	.525	.700	.662	.725	.700	.712					1.28	0.67	2.17	
53P(3)11+11	$\bar{X}_{53R}$	.462	.687	.725	.725	.737	.787							1.96I	
vs.	S^2_{53P}	.253	.213	.226	.202	.213	.207								
53R(3)11+11	S^2_{53R}	.252	.218	.202	.202	.196	.169								H
	S^2_{Pooled}	.252	.215	.214	.202	.204	.188								

$F1_{(10,70)} = 2.24$ and $^*F2_{(10,149)} = 2.15$ at $P = .025$. $D_{L(10,80)} = 1.71$ and $D_{U(10,80)} = 2.28$ at $P = .05$.

$F1_{(6,74)} = 2.59$ and $^*F2_{(6,153)} = 2.51$ at $P = .025$. $D_{L(6,80)} = 1.83$ and $D_{U(6,80)} = 2.16$ at $P = .05$.

Table 5.1 (cont'd.)

Groups		Time in Days 1	2	3	4	F1	F2	D	Box's Test
	$\bar{X}_{52P}$	.475	.512	.550	.537	2.01	0.97	2.19	
52P(3)7+13	$\bar{X}_{52R}$	.512	.625	.662	.687			2.17	
vs.	S^2_{52P}	.253	.253	.251	.252				
52R(3)7+13	S^2_{52R}	.253	.237	.226	.218				H
	S^2_{Pooled}	.253	.245	.239	.235				
	$\bar{X}_{54P}$	.487	.625	.562	.587	2.74	1.22	2.35	
54P(3)11+13	$\bar{X}_{54R}$	.512	.687	.662	.750			2.31	
vs.	S^2_{54P}	.253	.237	.249	.245				
54R(3)11+13	S^2_{54R}	.253	.218	.226	.196				H
	S^2_{Pooled}	.253	.227	.238	.218				
	$\bar{X}_{55P}$	.500	.537	.500	.500	2.49	0.96	2.30	
55P(3)13+13	$\bar{X}_{55R}$	.525	.525	.550	.587			2.31	
vs.	S^2_{55P}	.253	.252	.253	.253				
55R(3)13+13	S^2_{55R}	.253	.253	.251	.245				H
	S^2_{Pooled}	.253	.252	.252	.249				

$'F1_{(4,76)}=2.96$ and $^{*}F2_{(4,155)}= 2.88$ at P = .025.

$D_L = 1.89$ and $D_U = 2.10$ at P = .05.

taking place we would expect either significant attraction or repulsion, when in fact, a random distribution is exhibited.

If we examine Figure 5.2 (A & B), however, there is a trend for increased pairing with time. Table 5.2 shows the results of Hotelling's One-Sample comparison of each of these preference curves with an expected vector whose entries are all 0.5. The F-values show that in only three groups does aggregation become significantly different from random over time, namely in the 7+7, 7+11, and 11+11 situations. Whenever a 13 day old larva was a member of the pair grouping never reaches significance. This may be an indication that some sort of animal-animal interaction occurs over time. However, the preference vectors in Figure 5.2 and compared in Table 5.2 are unequal in time. Therefore, an analysis was performed using the first four days and the last four days of the experimental periods, the two being the same for 13 day old response vectors. The results of this analysis are presented in Table 5.3. It can be seen that the same groups show significant pairing except that the 7+7 group does not show significant pairing until the end of the experimental period, that is until the larvae have been in the experimental situation for at least four days.

One might contend that these differences are a function of time in the experimental situation. That is, 13 day old larvae do not show grouping behavior because they only have four days in the experimental apparatus, whereas a 7 day old larva has 10 days, and an 11 day old larva has 6 days. This may be true of the 7+7 situation, but it will be noted that 7+11 and 11+11 groups significantly pair

Figure 5.2

Mean response vectors for percent pairs of larvae on any food lump when the food lumps were initially unconditioned.

A. Pairs composed of larvae of the same age.

B. Pairs composed of larvae of different ages.

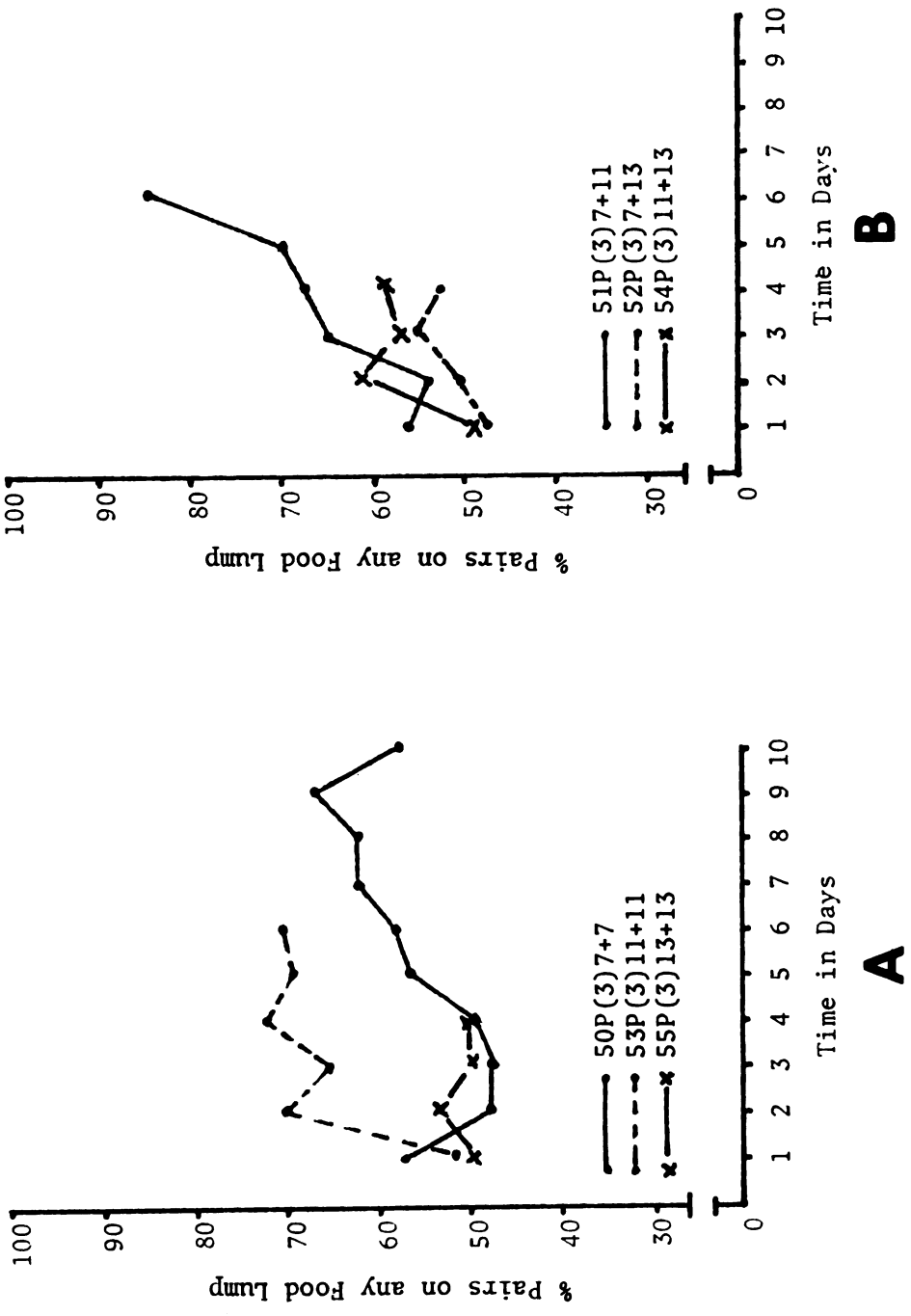

% Pairs on any Food Lump
100
90
80
70
60
50
40
30
0
1 2 3 4 5 6 7 8 9 10
Time in Days
50P(3)7+7
53P(3)11+11
55P(3)13+13
A
51P(3)7+11
52P(3)7+13
54P(3)11+13
B

Figure 5.2

Table 5.2

Results of Hotelling's One Sample comparison of each experimental (P) group in Figure 5.1 with an expected vector whose entries are all 0.5 to see if grouping (pairing), regardless of food lump, is different from random in the absence of homotypic conditioning. Response mean vectors($\bar{X}$), estimated variance vectors(S^2), and D-values for serial correlation are found in Table 5.1.

Groups	F
50P(3)7+7	2.81**
51P(3)7+11	13.32***
52P(3)7+13	0.57
53P(3)11+11	4.19**
54P(3)11+13	2.01
55P(3)13+13	0.20

** $F_{(10,70)}=2.59$, $F_{(6,74)}=3.08$, $F_{(4,76)}=3.59$ at P=.01.

*** $F_{(10,70)}=3.48$, $F_{(6,74)}=4.26$, $F_{(4,76)}=5.18$ at P=.001.

Table 5.3

Results of Hotelling's One Sample comparison of pairs, regardless of food lump, versus an expected vector whose entries are all 0.5. These comparisons are shown for the first four days and the last four days of each experimental group to see if time in the experimental situation affects grouping behavior.

Groups	First 4 Days F	Last 4 Days F
50P(3)7+7	1.07	2.99*
51P(3)7+11	3.53*	19.64***
52P(3)7+13	0.57	0.57
53P(3)11+11	5.83***	6.02***
54P(3)11+13	2.01	2.01
55P(3)13+13	0.20	0.20

*$F_{(4,76)} = 2.96$ at $P = .025$.

***$F_{(4,76)} = 5.18$ at $P = .001$.

when just the first four days are considered which is the same length of time 13 day old larvae have.

In Table 5.4 are presented the results of Hotelling's One- and Two-Sample comparisons for all possible comparisons of the experimental groups in Figure 5.1 for the first and last four days of the experimental periods. The F2-values show only one significant difference for the first four days, namely that 11+11 pairs are more frequently found than are 7+7 pairs. For the last four days, however, there are many more differences, the greatest pairing occurring when an 11 day old larva is a member of the pair, and the greatest difference is the 7+11 versus the 13+13 situation. Again illustrated is the fact that pairing is not significant when at least one member of the pair is a 13 day old larva. The F1-values in Table 5.4 point out some other differences, probably as a result of the heterogeneous variances in the Two-Sample case.

There are four possible ways to explain why aggregation may be occurring over time: (1) there may have been discrepancies between the two plain food lumps due to handling or nutritional differences, (2) the larvae may be responding to conditioning of other larvae, (3) some sort of larval-larval interaction may develop, and (4) combinations of 1-3 are possible.

We have already seen that initial preference for the food lumps is random, indication that the food lumps were equal in all respects. However, if the food lumps are actually not different, then pairing with respect to one food lump should be random, since the larval distribution should not be a function of food. To determine

Table 5.4

Results of Hotelling's One(') and Two(*) Sample comparisons among the experimental groups in Figure 5.1. Comparisons are made for the first 4 days and the last 4 days to see if time in the experimental situation affects grouping, and all comparisons are for pairs of larvae regardless of food lump. For the One Sample(') Test the groups used for the expected vectors are in the column headed EV. and those whose covariance matrix was used are in the column headed CV.

	FIRST FOUR DAYS			
Comparisons				
EV.	CV.	F1	F2	Box's Test	T^2 Test for Parallelism
50P(3)7+7 vs.	51P(3)7+11	3.76''	1.94	H	P
52P(#)7+13 vs.	50P(3)7+7	2.65	1.14	H	P
50P(3)7+7 vs.	53P(3)11+11		3.67**	H	
50P(3)7+7 vs.	54P(3)11+13	4.17''	2.22	H	P
50P(3)7+7 vs.	55P(3)13+13		0.69		P
52P(3)7+13 vs.	51P(3)7+11	2.71	1.42	H	P
51P(3)7+11 vs.	53P(3)11+11		1.66		P
51P(3)7+11 vs.	54P(3)11+13		2.23		P
55P(3)13+13 vs.	51P(3)7+11	4.06''	2.09	H	P
52P(3)7+13 vs.	53P(3)11+11	5.34'''	2.23	H	P
52P(3)7+13 vs.	54P(3)11+13		1.33		P
52P(3)7+13 vs.	55P(3)13+13	0.66	0.45	H	P
54P(3)11+13 vs.	53P(3)11+11	1.95	0.84	H	P
53P(3)11+11 vs.	55P(3)13+13		2.44		P
55P(3)13+13 vs.	54P(3)11+13	1.05	0.49	H	P

' $F1_{(4,76)} = 2.96$ and * $F2_{(4,155)} = 2.88$ at P = .025.

'' $F1_{(4,76)} = 3.59$ and ** $F2_{(4,155)} = 3.47$ at P = .01.

''' $F1_{(4,76)} = 5.18$ and *** $F2_{(4,155)} = 4.94$ at P = .001.

H Heterogeneous variances ($P \leq .05$).

P Mean response vectors are parallel.

Table 5.4 (cont'd.)

Comparisons EV.	CV.	F1	F2	Box's Test	T^2 Test for Parallelism
		LAST FOUR DAYS			
50P(3)7+7 vs.	51P(3)7+11		5.97***	H	
52P(3)7+13 vs.	50P(3)7+7		1.23		P
50P(3)7+7 vs.	53P(3)11+11	4.47''	1.50	H	P
50P(3)7+7 vs.	54P(3)11+13		2.07		P
55P(3)13+13 vs.	50P(3)7+7	3.41''	1.86	H	P
52P(3)7+13 vs.	51P(3)7+11		5.93***	H	P
51P(3)7+11 vs.	53P(3)11+11		2.91*	H	P
51P(3)7+11 vs.	54P(3)11+13		5.50***	H	
55P(3)13+13 vs.	51P(3)7+11		7.87***	H	
52P(3)7+13 vs.	53P(3)11+11	4.59''	2.34	H	P
52P(3)7+13 vs.	54P(3)11+13		1.33		P
55P(3)13+13 vs.	52P(3)7+13	0.66	0.45	H	P
53P(3)11+11 vs.	54P(3)11+13	3.10'	1.39	H	P
55P(3)13+13 vs.	53P(3)11+11	4.82''	2.16	H	P
55P(3)13+13 vs.	54P(3)11+13	1.05	0.49	H	P

' $F1_{(4,76)} = 2.96$ and * $F2_{(4,155)} = 2.88$ at P = .025.

'' $F1_{(4,76)} = 3.59$ and ** $F2_{(4,155)} = 3.47$ at P = .01.

''' $F1_{(4,76)} = 5.18$ and *** $F2_{(4,155)} = 4.94$ at P = .001.

H Heterogeneous variances ($P \leq .05$).

P Mean response vectors are parallel.

if larvae are pairing with respect to only food several contingency tables with Chi-square analyses were set up in which pairs on one food lump are compared with pairs on the other lump at each time point as well as for the total experimental period. This enables parcelling out the contribution of each time point in respect to pairing. If significance at any one or more time points results or if the Chi-square values are significant then the indication is that the two food lumps were different, even initially. This analysis is presented in Table 5.5.

It can be seen from these results that there are no significant Chi-square values either in each box or the totals. It may be concluded from this that pairing over time is not due to any discrepancies between food lumps. However, once the larvae select a food lump, they begin conditioning it and this conditioning may attract the other larva and thus pairing increases. For example, in a 7+13 situation the 13 day old larva may be doing more conditioning than the 7 and this attracts the 7 to where the 13 is located. We have already seen that whenever a 13 day old larva is present no significant pairing occurs (Tables 5.3 and 5.4) and examination of Figure 5.2 shows the response curve to be random. However, the 7+7, 11+11, and 7+11 situations show significant pairing with time. In the 7+7 and 11+11 situations we would expect each larva to be doing an equal amount of conditioning and thus excess conditioning by one larva which attracts the other larva is probably not the mechanism. It was also noted (Tables 5.3 and 5.4) that 7+7 day old larvae take longer to exhibit pairing than do the 7+11 or 11+11 situations and

Table 5.5

Contigency Tables with Chi-Square Analysis for each group in Figure 5.1 with the total Chi-Square partitioned to show the contribution of each time point for each food lump to the total. Within each box are shown the total number of pairs of larvae for each food lump at each time point and their individual Chi-Square values. Also shown are the total pairs at each time point or food lump and their Chi-Square contribution and the total Chi-Square value over all time points and both food lumps.

7+7

	t1	t2	t3	t4	t5	t6	t7	t8	t9	t10	
Lump 1	19 0.1186	15 0.0669	14 0.0377	14 0.0111	16 0.0570	16 0.1050	18 0.0365	17 0.1778	20 0.0020	19 0.1563	168 0.7671
Lump 2	27 0.1399	23 0.0417	22 0.0200	25 0.0060	30 0.0279	31 0.0618	33 0.0200	34 0.1050	34 0.0001	28 0.0913	287 0.5107
	46 0.2585	38 0.1086	36 0.0577	39 0.0171	46 0.0849	47 0.1668	51 0.0565	51 0.2828	54 0.0003	47 0.2476	455 1.2808

11+11

	t1	t2	t3	t4	t5	t6	
Lump 1	23 0.1950	28 0.0094	27 0.00003	29 0.0098	28 0.0094	29 0.0003	164 0.2239
Lump 2	19 0.1242	28 0.0102	26 0.0000	29 0.0106	28 0.0102	28 0.00005	158 0.1552
	42 0.3192	56 0.0196	53 0.00003	58 0.0204	56 0.0196	57 0.00008	322 0.3791

$^{*}X^2_{(1)} = 3.81$ at P - .05. $^{*}X^2_{(5)} = 11.07$ and $X^2_{(9)} = 16.91$ at P = .05.

Table 5.5 (cont'd.)

		t1	t2	t3	t4	t5	t6	
7+11	Lump 1	23 0.0400	21 0.0004	24 0.0359	27 0.0155	27 0.0007	32 0.0117	154 0.1042
	Lump 2	23 0.0361	23 0.0005	28 0.0333	28 0.0130	29 0.0005	36 0.0112	167 0.0947
		46 0.0761	44 0.0009	52 0.0692	55 0.0285	56 0.0013	68 0.0229	321 0.1989

		t1	t2	t3	t4	
13+13	Lump 1	20 0.5566	19 0.3620	15 0.2200	15 0.2200	69 1.3586
	Lump 2	20 0.4060	24 0.0157	25 0.1632	25 0.1632	94 0.7481
		40 0.9626	43 0.3777	40 0.3832	40 0.3832	163 2.1067

		t1	t2	t3	t4	
7+13	Lump 1	20 0.0526	19 0.1097	23 0.0454	21 0.0116	83 0.2229
	Lump 2	18 0.0526	22 0.1097	21 0.0454	22 0.0116	83 0.2229
		38 0.1052	41 0.2194	44 0.0908	43 0.0232	166 0.4458

		t1	t2	t3	t4	
11+13	Lump 1	12 0.1365	18 0.0452	16 0.0225	16 0.0005	62 0.2047
	Lump 2	27 0.0721	32 0.0230	29 0.0113	31 0.0003	119 0.1067
		39 0.2086	50 0.0682	45 0.0338	47 0.0008	181 0.3114

*$X^2_{(1)} = 3.84$, $X^2_{(3)} = 7.81$, and $X^2_{(5)} = 11.07$ at P = .05.

that the 7+11 group exhibits the greatest amount of pairing. Since initial preference in relation to food lump is random, we would not expect Table 5.5 to shed any light on why pairing is occurring over time, even if the larvae are changing those food lumps and making them more "attractive" to the other member of the pair. However, we can approach the question of whether something one larva did to one food lump is attracting the other larva in another way.

Let us hypothesize that the older larvae do more conditioning than the younger larvae over time and that the younger larvae are attracted to that conditioning. We can test this hypothesis using the 7+11, 11+13, and 7+13 situations. In these groups every dish in which the older larva initially selected one of the plain food lumps and remained there for the whole experiment was selected for analysis. There were 60 such dishes in the 7+11 group, 68 in the 7+13 group, and 65 in the 11+13 group. Those food lumps are now designated as the conditioned lump and we can ask how often the younger larva is attracted to that lump, either because of the presence of the other larva or the conditioning. The results of this analysis are in Table 5.6 and graphed in Figure 5.3.

The results demonstrate that the 7 day old larva is homing in on the lump with the 11 day old larva in it, but that neither a 7 or 11 day old larva are attracted to the lump with the 13 day old larva in it. It will also be noted that the response vectors in Figure 5.3 are very similar to those in Figure 5.2 B in which pairs over time were initially graphed.

Table 5.6

Results of Hotelling's One-Sample analysis on the preference of the younger larva of a pair for the food lump in which the older larva initially selected and remained in for the entire experimental period. Comparison is made against an expected vector whose entries are all 0.5.

Group	F1
54P(5)11+13	2.10
52P(5)7+13	2.26
5.P(5)7+11	10.27"

'$F1_{(4,65)} = 3.00$, $F1_{(4,68)} = 2.99$, and $F1_{(4,61)} = 2.63$ at $P = .025$.

"$F1_{(6,61)} = 4.37$ at $P = .001$.

Of the 12 dishes in group 52P(5)7+13 in which the 13 did not initially select and remain in a food lump, in 5 dishes the 7 moved away when the 13 moved to its lump and in 3 dishes the 13 moved away when the 7 moved to its lump. In group 54P(5)11+13, of the 19 dishes in which the 11 did not stay in its initial choice, the 7 moved away 3 times when the 11 moved to its lump and the 11 moved away 4 times when a 7 moved to it. In group 54P(5)11+13 of the 15 dishes in which the 13 did not choose and stay in a lump, in 5 dishes the 11 moved away when the 13 moved to its lump and in 4 dishes the 13 moved away when the 11 moved to its lump.

From the results of Experiment I it is possible to develop the following hypothesis. Only pairs 7+7, 11+11, and 7+11 are significant over time and whenever a 13 day old larva is one of the test larvae, pairing does not occur, regardless of the age of the other larva. In the cases where significant grouping is found it is

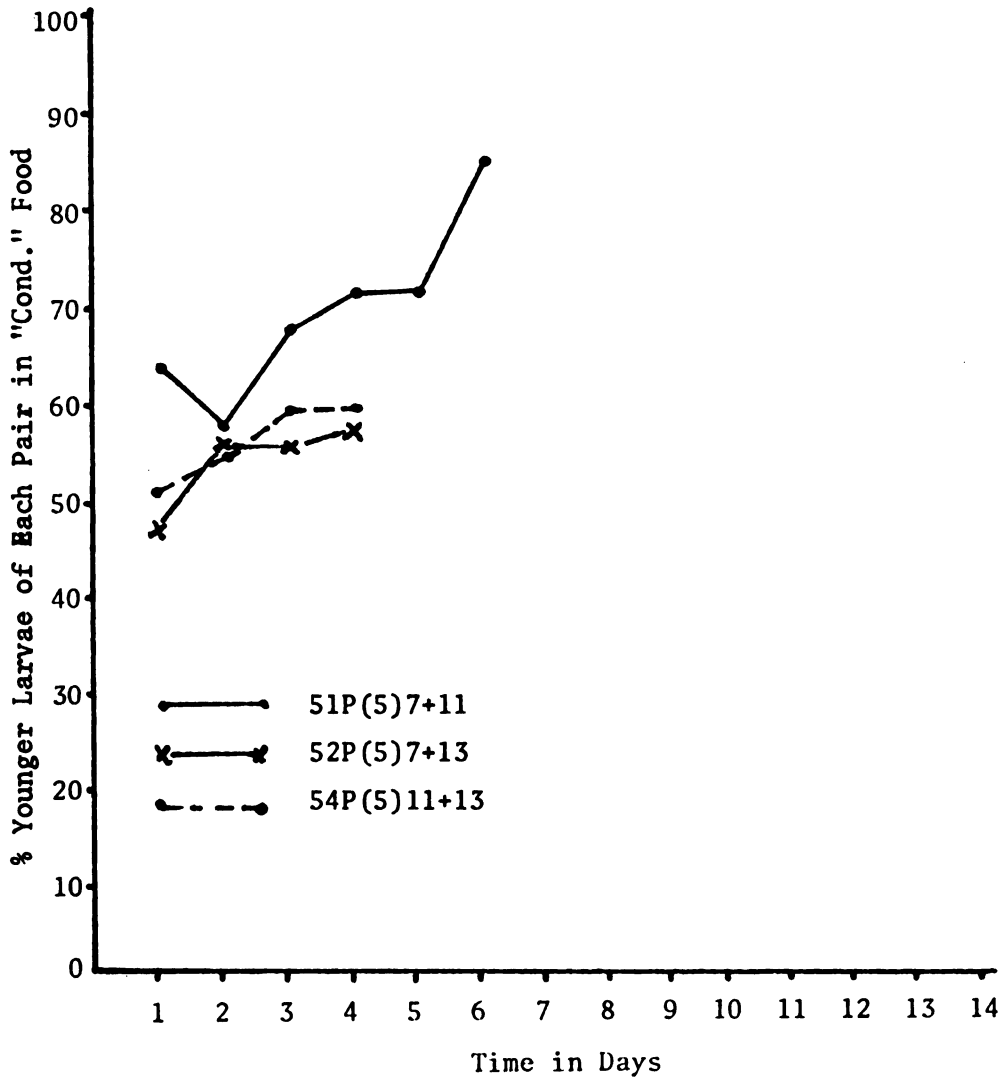


Figure 5.3

Mean response vectors of the younger larvae in a pair to a particular "plain" food lump that was initially selected by the older member of the pair and in which the older larva remained in for the entire experimental period. That food lump is considered the "conditioned" lump for purposes of these curves.

not because of some difference between the two food lumps, rather it appears to be due to the presence of another larva or to something that larva does to the food. When it was possible to distinguish individuals, it was found that 7 day old larvae would move to where an 11 day old larva was and stay there. There are two possibilities for this occurrence: either the younger larva prefers to be with the other larva, or the other larva, being older and bigger, is more highly conditioning its food lump than is the 7 day old larva and this attracts the 7. However, there would also appear to be an animal-animal interaction in this behavior since neither a 7 or 11 is so attracted to where a 13 day old larva's lump is the the 13 should be doing the most conditioning. It is possible that the 13 day old larva is doing a different kind of conditioning that younger larvae do not find as attractive. However, the response of 7 and 11 day old isolates, Chapter IV, to similar degrees of conditioning are very strong, indicating there is not a qualitative conditioning difference.

In situations where both larvae are the same age, 7+7, 11+11, and 13+13 it is not possible to definitively determine what is going on. The initial 7+7 and 11+11 situations exhibit significant grouping with time, the 13+13 does not. It would appear that a 13 day old larva will not tolerate the presence of any other larva, but younger larvae will. However, both 7 day old larvae should be equally conditioning their environments as should both 11 day old larvae. One possible explanation for grouping in these situations is on the basis of animal-animal interactions not conditioning. That is, the presence of older larvae overrides the preference for conditioning by younger

larvae, whereas the presence of a younger larva does not. We will come back to these points in the discussion section.

To be sure that any one food lump was sufficient for development of two larvae of any age and that the distributions observed in my experiments are not due to food limitation, the following was done. One hundred and twenty dishes were set up with one food lump in each dish. In 60 of these dishes the lump was highly conditioned (the maximum conditioning of any experiment in this paper) and in 60 dishes it was plain. Two 7 day old larvae, two 11 day old larvae, and two 13 day old larvae were raised in 20 dishes in each situation and their pupation time measured. If the developmental time of these larvae is not different among the groups then food was not a limiting factor in any of the experiments presented in this paper. The results in Table 5.7 show that development was not different from group to group.

Table 5.7

Analysis of Variance on developmental times of two 7, two 11, and two 13 day old larvae reared on single conditioned or plain food lumps.

Source of Variation	d.f.	S.S.	M.S.	F ratio
Between Group	5	3.5416	0.7083	0.9723
Within Group	114	83.05	0.7285	
Total	119	86.5916		

$F_{(5,119)} = 2.2914$ at $P = .05$.

Experiment II: When Initial Conditioning is Present, is Preference for Conditioning Affected by the Presence of a Conspecific, the Age of the Conspecific, and Whether the Conspecific is a Resident in the Conditioned Food?

These experiments examine whether the preference of a larva for conditioning is affected by the presence of a conspecific when that conspecific is an established resident in the conditioned lump or when it is introduced with the other larva. The experimental designs for these experiments are shown in Figures 5.4 and 5.5. Each experimental group has a sample size of 80 and the identification numbers were explained earlier.

Only two ages of test larvae are used, 7+13 days old, and only two degrees of conditioning, high and low. Isolate larvae, of all ages, are attracted to both degrees of conditioning (see Chapter IV), but at a lower level to the low conditioning than to the high conditioning. These two degrees of conditioning, namely LOW= 1,12,(7,13) and HIGH=7,6,(7,13) were chosen because the conditioning larva is 13 days old at the end of the conditioning period in both groups and may then be left in the conditioned food as a resident larva for some of the experiments. To maximize the probability of finding animal-animal interactions only 7 and 13 day old larvae are utilized throughout the remainder of these studies.

The experimental procedure for Figure 5.4 is as for all previous experiments. When the conditioning period is over, for each degree of conditioning, the conditioning larvae are removed and a pair of test larvae added, the pairs being either 7+7, 7+13, or 13+13. In

		LOW CONDITIONING		HIGH CONDITIONING	
		Age of Test Larva		Age of Test Larva	
		7	13	7	13
Age of Test Larva	7	56PL(1-3)7+7 2/5/75	57PL(1-5)7+13 2/5/75	63PH(1-3)7+7 2/5/75	64PH(1-5)7+13 2/5/75
	13		58PL(1-3)13+13 2/5/75		65PH(1-3)13+13 2/5/75
		59IL(1)7 2/5/75	60IL(1)13 2/5/75	66IH(1)7 2/5/75	67IH(1)13 2/5/75

Figure 5.4

Experimental design (top) for animal-animal interactions, at two levels of conditioning, when the conditioning larva is removed and both test larvae are new to the experimental situation.

		LOW COND.	HIGH COND.
		Age of Test Larva	
		7	13
Age of Test Larva	7	61PL(1-5)7+13 2/5/75	68PH(1-5)7+13 2/5/75
	13	62PL(1-3)13+13 2/5/75	69PH(1-3)13+13 2/5/75

Figure 5.5

Experimental design (left) for the effect of a resident larva on the preference of a test larva for biological conditioning. The resident larva is the original conditioning larva.

Figure 5.5, however, at the end of the conditioning period the conditioning larva is left in the conditioned lump and a single 7 or 13 day old larva added. Both experiments utilize two test larvae, but in one, both are new to the situation and in the other, one is a resident (always a 13 day old larva) and the other is new to the situation (either a 7 or 13 day old larva).

Table 5.8 lists each group from Figures 5.4 and 5.5 which have been analyzed, their mean response (preference), their estimated variance (S^2), and the D-values for serial correlation. It must be remembered that there are several ways in which to analyze any one experimental group based on (1) total larvae in the conditioned lump, (2) pairs in the conditioned lump, (3) pairs regardless of lump, (4) only the 13 day old larva in the conditioned lump and (5) only the 7 day old larva in the conditioned lump. The groups are listed as such in Table 5.8. However, not all of the comparisons were made because they were not necessary to answer the questions being asked and some comparisons would be meaningless.

To find out if larvae in pairs are attracted to low and high conditioning, each group was compared to an expected vector whose entries are all 0.5. This analysis used the total number of larvae on conditioned food to determine if two larvae distribute themselves randomly or are attracted to the conditioned food.

The results in Table 5.9 demonstrate that, regardless of degree of conditioning or age combination of larvae, all groups are significantly different from random and are attracted to conditioned food as can be seen by examining their mean response vectors in

Table 5.8

This table presents the response mean vectors($\bar{X}$), estimated variance vectors(S^2), and D-values for serial correlation for the groups in Figures 5.3 and 5.4 to be used in future comparisons.

LOW CONDITIONING

Groups		Time in Days 1	2	3	4	5	6	7	8	9	10	D
56PL(1)7+7	$\bar{X}$	.637	.643	.650	.643	.643	.712	.781	.743	.781	.681	1.46*
	S^2	.120	.134	.142	.134	.147	.119	.113	.139	.132	.115	
56PL(2)7+7	$\bar{X}$	.412	.450	.475	.450	.475	.537	.662	.637	.575	.375	1.46*
	S^2	.245	.251	.253	.251	.253	.252	.226	.234	.247	.237	
57PL(1)7+13	$\bar{X}$	.562	.675	.700	.681							2.20
	S^2	.123	.115	.099	.109							
57PL(1)7+13 Wandering	$\bar{X}$	.562	.675	.700	.681	.718	.612	.531	.443	.431	.375	1.60*
	S^2	.123	.115	.099	.109	.119	.133	.091	.076	.068	.092	
57PL(2)7+13	$\bar{X}$	.312	.450	.475	.462							2.20
	S^2	.218	.251	.253	.252							
57PL(4)7+13	$\bar{X}$	.650	.725	.775	.762							2.11
	S^2	.230	.202	.177	.183							
57PL(5)7+13	$\bar{X}$	.475	.625	.625	.600	.687	.750	.775	.750	.750	.625	1.40*
	S^2	.253	.237	.237	.243	.218	.190	.177	.190	.190	.237	
58PL(1)13+13	$\bar{X}$	.837	.856	.856	.825							2.39
	S^2	.075	.071	.058	.077							
58PL(2)13+13	$\bar{X}$	.737	.750	.725	.687							2.25
	S^2	.196	.190	.202	.218							
59IL(1)7	$\bar{X}$	.537	.550	.660	.662	.737	.725	.750	.725	.687	.525	1.39*
	S^2	.252	.251	.226	.226	.196	.202	.190	.202	.218	.253	
60IL(1)13	$\bar{X}$	.750	.775	.787	.837							2.31
	S^2	.190	.177	.169	.138							
59IL(1)7 + 60IL(1)13/2	$\bar{X}$	.643	.662	.725	.750	.781	.706	.562	.437	.418	.337	
61PL(1)7+13	$\bar{X}$	.843	.806	.787	.798							2.24
	S^2	.054	.060	.062	.061							
61PL(2)7+13	$\bar{X}$	.687	.612	.575	.587							2.24
	S^2	.218	.240	.247	.245							
61PL(5)7+13	$\bar{X}$	.700	.637	.600	.625	.675	.775	.812	.850	.800	.612	1.43*
	S^2	.213	.234	.243	.237	.222	.177	.154	.129	.162	.240	
62PL(1)13+13	$\bar{X}$	.862	.893	.900	.875							1.96I
	S^2	.063	.049	.047	.073							
62PL(2)13+13	$\bar{X}$	.750	.812	.812	.800							2.06I
	S^2	.190	.154	.154	.162							

*$D_{L(10,80)}$ = 1.71 and $D_{U(10,80)}$ = 2.28 at P = .05.

*$D_{L(4,80)}$ = 1.89 and $D_{U(4,80)}$ = 2.10 at P = .05.

I D-value inconclusive,

Table 5.8 (cont'd.)

HIGH CONDITIONING

Groups		Time in Days 1	2	3	4	5	6	7	8	9	10	D
63PH(1)7+7	$\bar{X}$	.600	.693	.743	.743	.762	.750	.762	.768	.787	.768	1.26*
	S^2	.129	.092	.082	.082	.082	.101	.120	.120	.100	.120	
63PH(2)7+7	$\bar{X}$	.375	.450	.525	.525	.562	.575	.687	.700	.662	.650	1.30*
	S^2	.237	.251	.253	.253	.249	.247	.218	.213	.226	.230	
64PH(1)7+13	$\bar{X}$	.743	.706	.718	.712							2.03I
	S^2	.108	.099	.100	.119							
64PH(1)7+13 Wandering	$\bar{X}$	.743	.706	.718	.712	.750	.737	.506	.506	.525	.425	1.27*
	S^2	.108	.099	.100	.119	.108	.114	.092	.066	.063	.032	
64PH(2)7+13	$\bar{X}$	.575	.487	.512	.537							1.98I
	S^2	.247	.253	.253	.252							
64PH(4)7+13	$\bar{X}$	.862	.812	.837	.800							2.24
	S^2	.120	.154	.138	.162							
64PH(5)7+13	$\bar{X}$	.625	.600	.612	.637	.725	.787	.762	.825	.875	.850	1.27*
	S^2	.237	.243	.240	.234	.202	.169	.183	.146	.111	.129	
65PH(1)13+13	$\bar{X}$	.650	.750	.806	.806							2.12
	S^2	.135	.108	.079	.079							
65PH(2)13+13	$\bar{X}$	.462	.600	.650	.650							2.07I
	S^2	.252	.243	.230	.230							
66IH(1)7	$\bar{X}$	.737	.837	.887	.862	.875	.900	.900	.900	.925	.800	1.49*
	S^2	.196	.138	.101	.120	.111	.091	.091	.091	.070	.162	
67IH(1)13	$\bar{X}$	.912	.962	.962	.975							2.22
	S^2	.081	.037	.037	.025							
66IH(1)7 + 67IH(1)13	$\bar{X}$	.850	.900	.931	.918	.900	.868	.650	.581	.593	.531	
68PH(1)7+13	$\bar{X}$	.806	.837	.887	.912							1.98I
	S^2	.060	.068	.057	.043							
68PH(2)7+13	$\bar{X}$	.612	.712	.800	.837							1.85*
	S^2	.240	.207	.162	.138							
68PH(5)7+13	$\bar{X}$	.612	.750	.837	.875	.850	.787	.825	.900	.950	.887	1.55*
	S^2	.240	.190	.138	.111	.129	.169	.146	.091	.048	.101	
69PH(1)13+13	$\bar{X}$	.956	.918	.912	.900							2.27
	S^2	.020	.041	.043	.059							
69PH(2)13+13	$\bar{X}$	.912	.850	.837	.837							2.21
	S^2	.081	.025	.036	.011							

*$D_{L(10,80)}$ = 1.71 and $D_{U(10,80)}$ = 2.28 at P = .05. I D-value inconclusive.

*$D_{L(4,80)}$ = 1.89 and $D_{U(4,80)}$ = 2.10 at P = .05.

Table 5.9

Hotelling's One Sample test on the experimental groups in Figures 5.4 and 5.5. Total larvae in the conditioned food (paired, singly, or none) is compared with an expected vector whose entries are all 0.5. Response mean vectors($\bar{X}$), estimated variance vectors(S^2), and D-values for serial correlation are found in Table 5.8.

LOW CONDITIONING		HIGH CONDITIONING	
Group	F	Group	F
56PL(1)7+7	6.02*	63PH(1)7+7	9.50*
57PL(1)7+13	8.23*	64PH(1)7+13	12.98*
58PL(1)13+13	51.89*	65PH(1)13+13	23.83*
59IL(1)7	4.56*	66IH(1)7	23.75*
60IL(1)13	18.66*	67PIH(1)13	792.07*
61PL(1)7+13	63.53*	68PH(1)7+13	83.70*
62PL(1)13+13	325.50*	69PH(1)13+13	238.89*

*$F_{(10,70)} = 3.48$ and $F_{(4,76)} = 5.18$ at $P = .001$.

Table 5.8. It must be remembered, however, that when the resident groups are so analyzed, half of the larvae are already in the conditioned food. This will be sorted out shortly. It will be noted that the F-values for high conditioning are generally higher than for low conditioning indicating greater attraction to high than low conditioning as would have been predicted on the basis of response to conditioning alone.

Table 5.10 presents the results of the analysis on whether the number of pairs in conditioned food is significantly different from random. In every case aggregation is occurring in the conditioned food and more so in highly conditioned food. Therefore, not only are total larvae in conditioned food significantly different from random, but of those larvae there are more pairs than would be predicted on the basis of chance alone.

Neither of these analyses says anything about whether an individual's preference for the conditioned food has been altered by the presence of a conspecific. That is, are the interactions between the larvae affecting individual behavior? This may be answered by treating each pair of larvae as a unit and comparing it to a similar unit from the isolated situation or by comparing only one member of a pair with an isolate's behavior of the same age. For example, if animal-animal interaction has no effect on an individual's preference for conditioning, then the total number of 13 day old larvae, in the situations where two 13 day old larvae are present, in conditioned food, should not be different from the response vector of isolated 13 day old larvae; or the response vector for the 7+13 situation should

Table 5.10

Hotelling's One Sample test on the experimental groups in Figures 5.4 and 5.5. Pairs of larvae on conditioned food are compared with an expected vector whose entries are all 0.25. Response mean vectors($\bar{X}$), estimated variance vectors(S^2), and D-values for serial correlation are found in Table 5.8.

LOW CONDITIONING		HIGH CONDITIONING	
Group	F	Group	F
56PL(2)7+7	7.04**	63PH(2)7+7	8.74**
57PL(2)7+13	4.28*	64PH(2)7+13	11.74**
58PL(2)13+13	34.16**	65PH(2)13+13	13.77**
61PL(2)7+13	24.23**	68PH(2)7+13	49.44**
62PL(2)13+13	49.41**	69PH(2)13+13	145.96**

*$F_{(10,70)}$ 2.86 and $F_{(4,76)}$ = 4.11 at P = .005.

**$F_{(10,70)}$ = 3.48 and $F_{(4,76)}$ = 5.18 at P = .001.

not be different from the average response of a 7 day old isolate and a 13 day old isolate; or the response of a 7 day old larvae when with a 13 day old larva should not be different from that of a 7 day old isolate's response. Such comparisons have been made in Table 5.11 and graphed in Figures 5.6 and 5.7. Using Hotelling's Two-Sample approach it can be seen from Table 5.11 that there are no significant differences in response on the part of larvae, when grouped or isolated, to low conditioning, although the One-Sample method shows some differences. Examination of Figure 5.6 shows all the response curves for respective comparisons to be about the same. However, in every case the comparisons when high conditioning is used demonstrate that response of individual larvae when with a conspecific, regardless of age, is lower than when a similar aged larva is isolated. This is graphically demonstrated in Figure 5.7. It will also be noted that the greatest effect of animal-animal interaction is when at least one of the members of the pair is 13 days old. Therefore, animal-animal interactions lower individual preferences for conditioned food only when that food is highly conditioned. We will come back to these considerations later.

In Table 5.12 are found comparisons examining resident versus non-resident effects on individual preferences for low and high conditioning, and the mean vectors are graphed in Figures 5.8 and 5.9. Hotelling's Two-Sample test (F2-values in Table 5.12) shows that in low conditioning, whether one of the larvae is a 13 day old established resident in the conditioned food, or both larvae are new to the situation, it has no effect on individual preferences for conditioning.

Table 5.11

Hotelling's One(') and Two(*) Sample comparisons among the groups in Figure 5.4. Assuming no animal-animal interaction, the response vectors for two larvae of equal age should be the same as the response of one larva of the same age. Also the response vector for either larvae, when the two test larvae are unequal in age, should be the same as the response of one larvae of the appropriate age.

Comparisons EV.	CV.	LOW CONDITIONING F1	F2	Box's Test	T^2 Test for Parallelism
59IL(1)7	vs. 56PL(1)7+7	5.58'''	1.68	H	P
59IL(1)7 + 60IL(1)13/2	vs. 57PL(1)7+13	1.95			
57PL(4)7+13	vs. 60IL(1)13	2.57'	1.13	H	P
57PL(5)7+13	vs. 59IL(107	2.30'	1.20	H	P
58PL(1)13+13	vs. 60IL(1)13	14.11'''	2.81	H	

' $F1_{(10,70)} = 2.24$, and $F1_{(4,76)} = 2.96$, and * $F2_{(10,149)} = 2.15$, and $F2_{(4,155)} = 2.88$ at P = .025.

'' $F1_{(10,70)} = 2.59$, and $F1_{(4,76)} = 3.59$, and ** $F2_{(10,149)} = 2.46$, and $F2_{(4,155)} = 3.47$ at P = .01.

''' $F1_{(10,70)} = 3.48$, and $F1_{(4,76)} = 5.18$, and *** $F2_{(10,149)} = 3.24$, and $F2_{(4,155)} = 4.94$ at P = .001.

EV.= expected vector and CV.= covariance matrix for the One Sample comparisons.

H Heterogeneous variances ($P \leq .05$).

P Mean response vectors are parallel ($P \leq .05$).

Table 5.11 (cont'd.)

Comparisons EV.	CV.	HIGH CONDITIONING F1	F2	Box's Test	T^2 Test for Parallelism
63PH(1)7+7	vs. 66IH(1)7		2.38*	H	P
66IH(1)7 + 67IH(1)13/2	vs. 64PH(1)7+13	6.18'''			
64PH(4)7+13	vs. 67IH(1)13		3.29*	H	P
64PH(5)7+13	vs. 66IH(1)7		3.08**	H	
65PH(1)13+13	vs. 67IH(1)13		8.85***	H	P

* $F2_{(10,149)} = 2.15$ and $F2_{(4,155)} = 2.88$ at P = .025. ''' $F1_{(10,70)} = 3.48$ at P = .001.

** $F2_{(10,149)} = 2.46$ and $F2_{(4,155)} = 3.47$ at P = .01.

*** $F2_{(10,149)} = 3.24$ and $F2_{(4,155)} = 4.94$ at P = .001.

EV.= expected vector and CV.= covariance matrix for the One Sample comparisons.

H Heterogeneous variances ($P \leq .05$).

P Mean response vectors are parallel ($P \leq .05$).

Figure 5.6

Mean response vectors for comparisons (Table 5.11) of two larvae situations with one larva situations to find if individual preference for low conditioned food is affected by the presence of a conspecific.

A. 59IL(1)7 vs. 56PL(1)7+7

B. 60IL(1)13 vs. 58PL(1)13+13

C. $57PL(1)7+13 \text{ vs. } \frac{59IL(1)7 + 60IL(1)13}{2}$

D. 59IL(1)7 vs. 57PL(5)7+13

E. 57PL(4)7+13 vs. 60IL(1)13

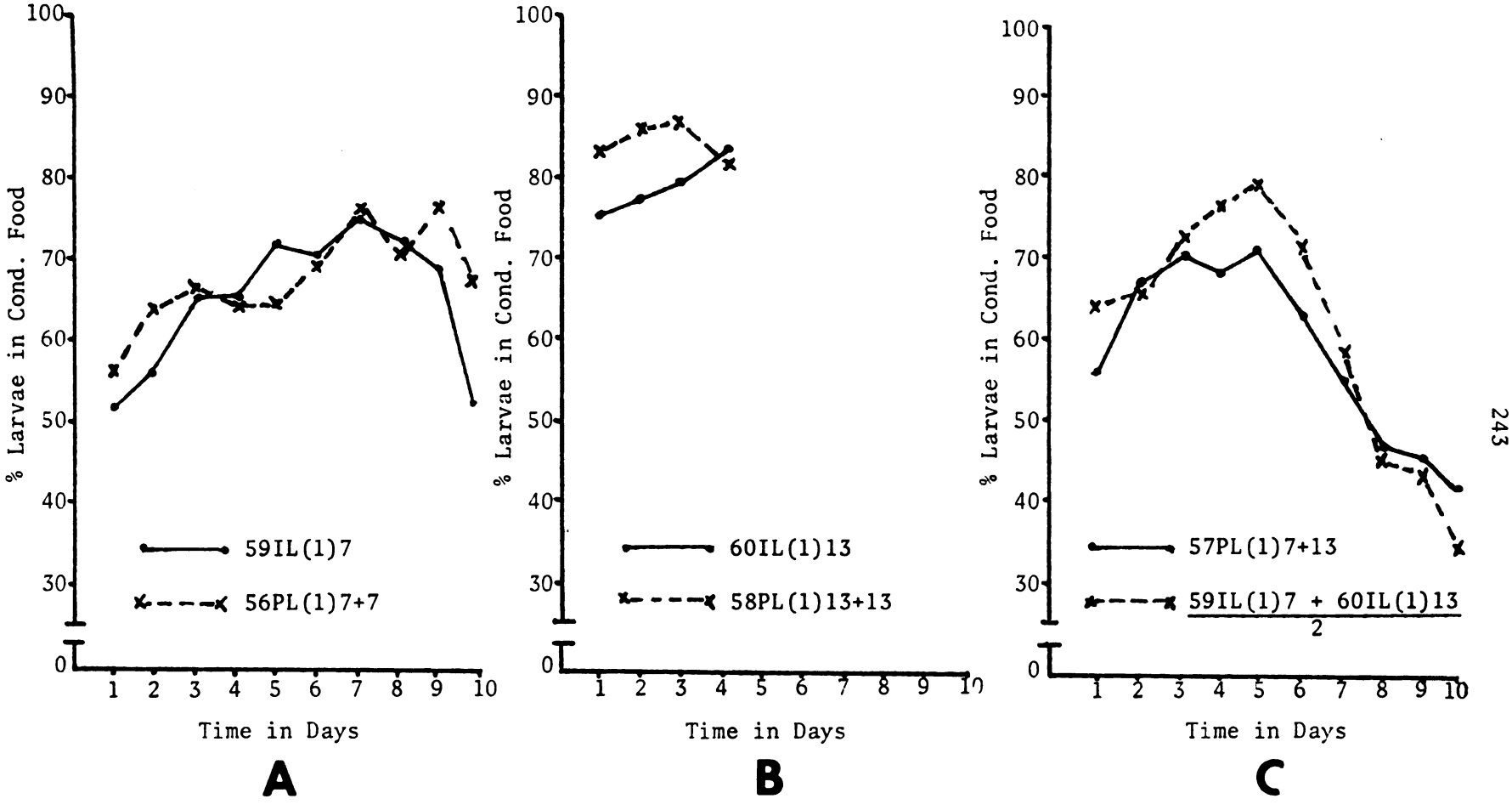
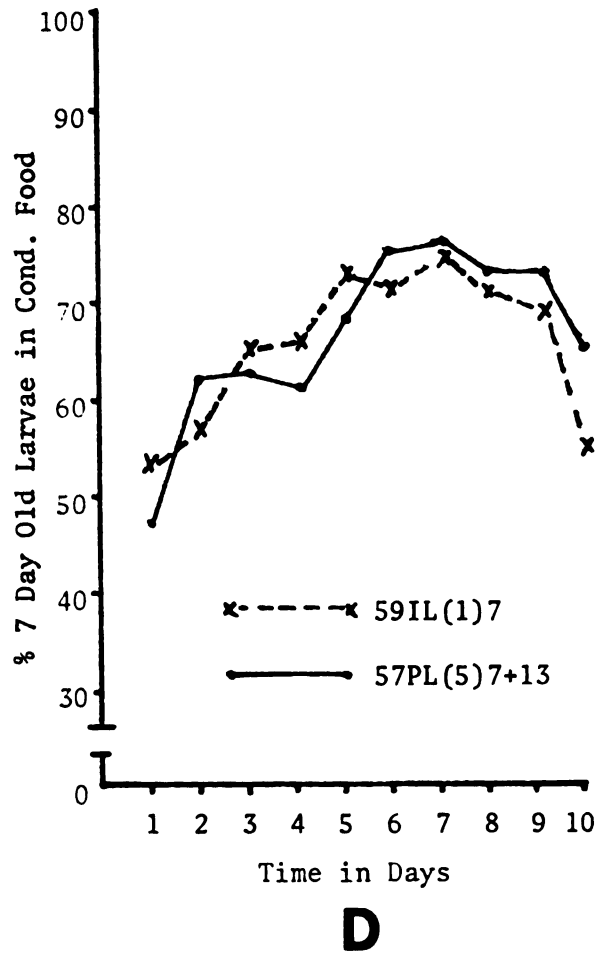

100
90
80
70
60
50
40
30
0
% Larvae in Cond. Food
1 2 3 4 5 6 7 8 9 10
Time in Days
59IL(1)7
56PL(1)7+7
A
60IL(1)13
58PL(1)13+13
B
57PL(1)7+13
59IL(1)7 + 60IL(1)13
2
C

Figure 5.6



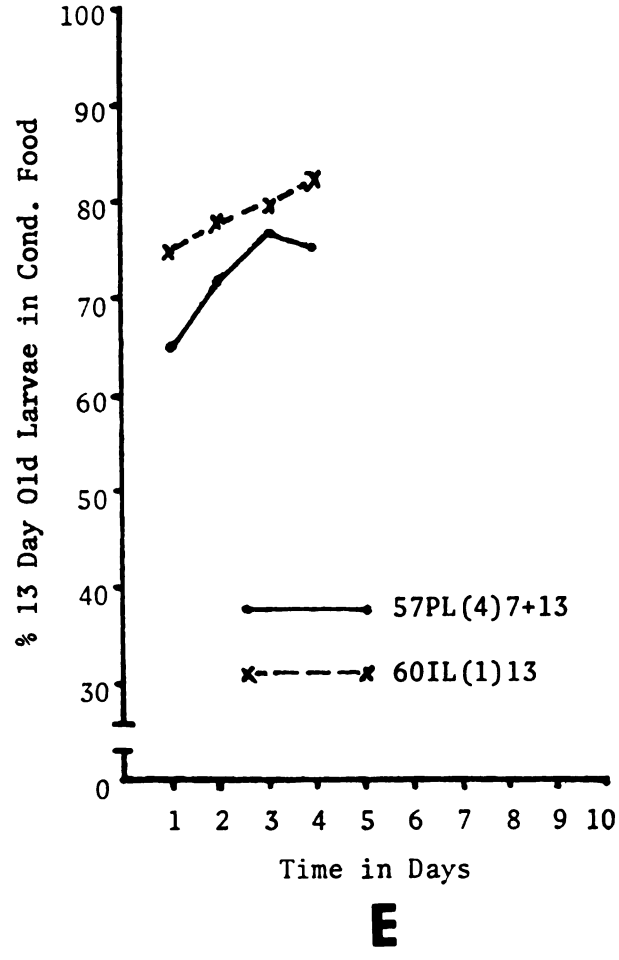


Figure 5.6 (cont'd.)

Figure 5.7

Mean response vectors for comparisons (Table 5.11) of two larvae situations with one larva situations to find if individual preference for high conditioned food is affected by the presence of a conspecific.

A. 66IH(1)7 vs. 63PH(1)7+7

B. 67IH(1)13 vs. 65PH(1)13+13

C. $64PH(1)7+13 \text{ vs. } \frac{66IH(1)7 + 67IH(1)13}{2}$

D. 66IH(1)7 vs. 64PH(5)7+13

E. 67IH(1)13 vs. 64PH(4)7+13

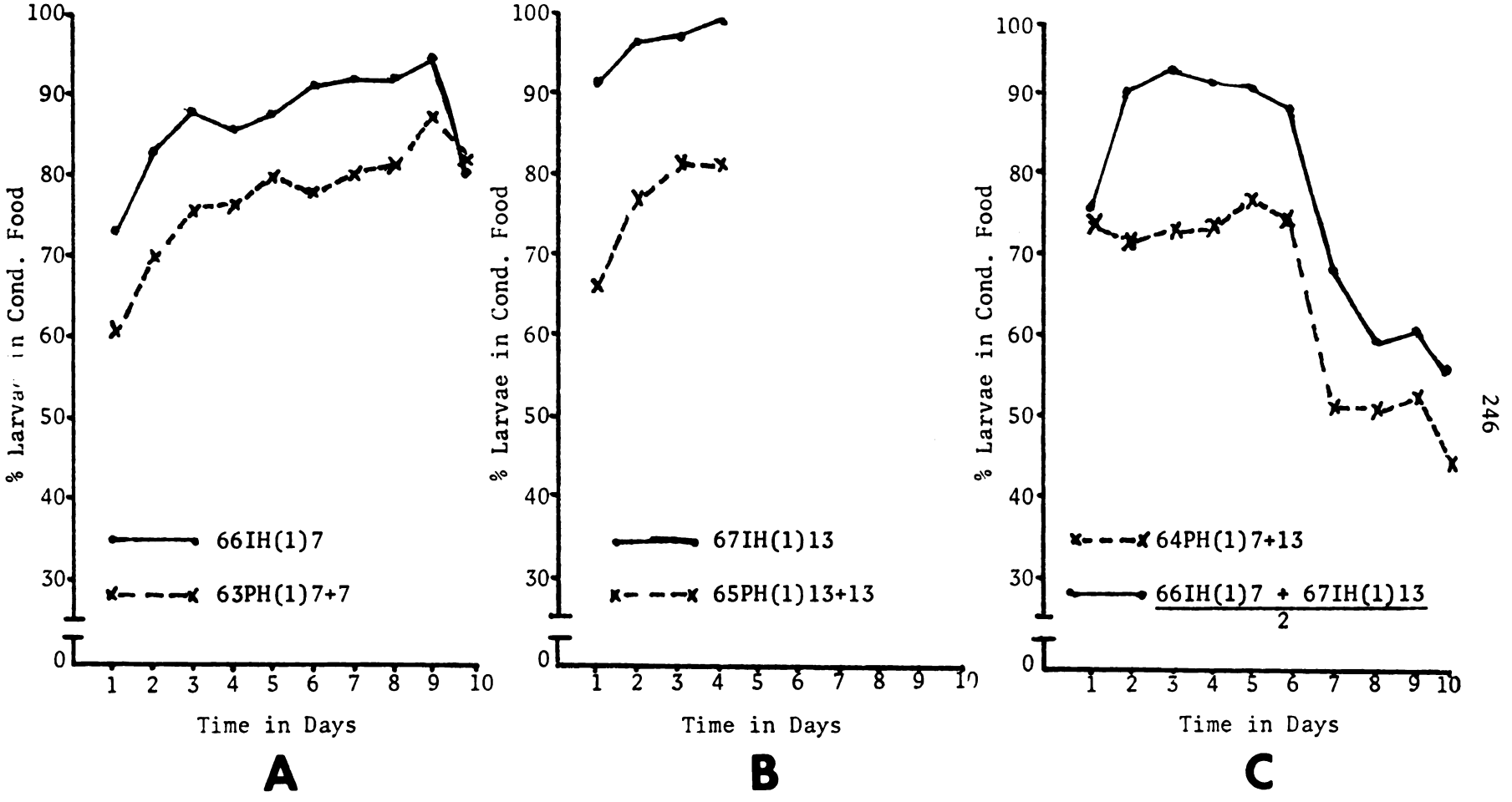
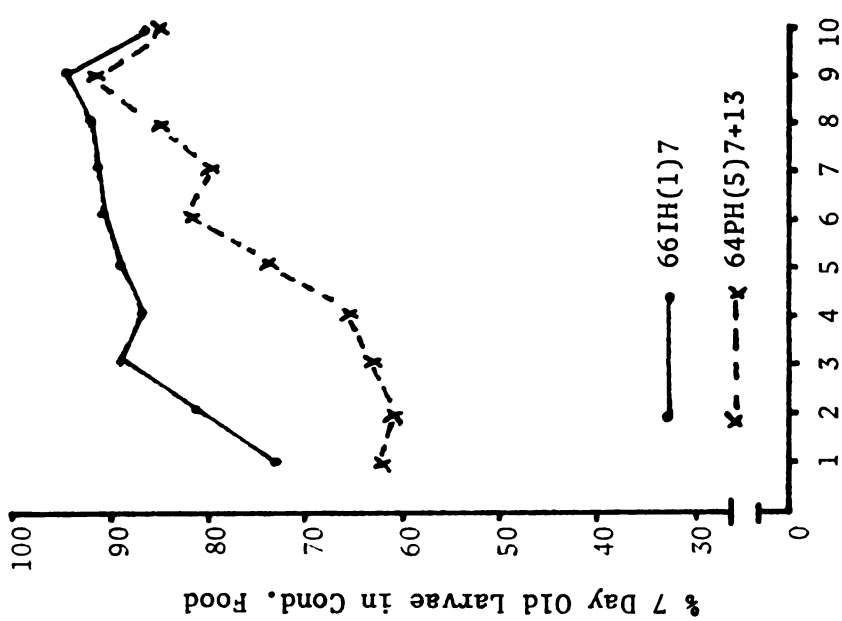

100
90
80
70
60
50
40
30
0
% Larvae in Cond. Food
66IH(1)7
63PH(1)7+7
1 2 3 4 5 6 7 8 9 10
Time in Days
A
67IH(1)13
65PH(1)13+13
B
64PH(1)7+13
66IH(1)7 + 67IH(1)13
2
C

Figure 5.7



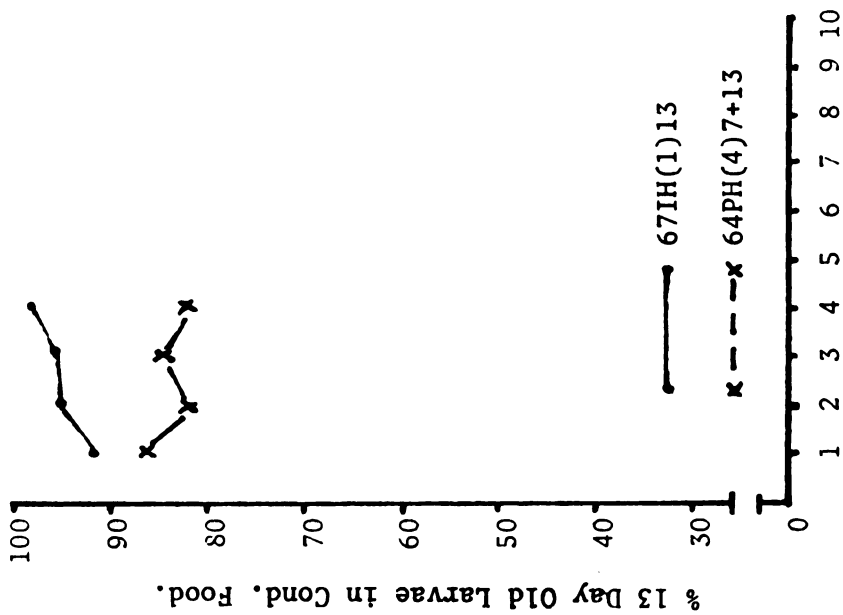


Figure 5.7 (cont'd.)

Table 5.12

Hotelling's One(') and Two(*) Sample comparisons among the experimental groups in Figures 5.4 and 5.5 to determine resident versus non-resident effects on preference for biological conditioning. Assuming no animal-animal interactions there should not be any differences in these comparisons. There should also not be any differences if the effect of a resident larva on the response larva is the same as the effect of another larva introduced simultaneously with the response larva.

Comparisons EV.	CV.	LOW CONDITIONING F1	F2	Box's Test	T^2 Test for Parallelism
61PL(5)7+13	vs. 57PL(5)7+13	8.99'''	1.35	H	P
59IL(1)7	vs. 61PL(5)7+13	7.06'''	2.01	H	P
59IL(1)7	vs. 57PL(5)7+13	2.30'	1.20	H	P
58PL(2)13+13	vs. 62PL(2)13+13		0.79		P

' $F1_{(10,70)} = 2.24$, and $F1_{(4,76)} = 2.96$, and * $F2_{(10,149)} = 2.15$, and $F2_{(4,155)} = 2.88$ at P = .025.

''' $F1_{(10,70)} = 3.48$, and $F1_{(4,76)} = 5.18$ at P = .001.

EV.= expected vector and CV.= covariance matrix for the One Sample comparisons.

H Heterogeneous variances ($P \leq .05$).

P Mean response vectors are parallel.

Table 5.12 (cont'd.)

Comparisons EV.	CV.	HIGH CONDITIONING F1	F2	Box's Test	T^2 Test for Parallelism
68PH(5)7+13	vs. 64PH(5)7+13		2.38*	H	
68PH(5)7+13	vs. 66IH(1)7	5.68'''	2.02	H	
64PH(5)7+13	vs. 66IH(1)7		3.08*	H	
69PH(2)13+13	vs. 65PH(2)13+13		12.73***	H	

' $F1_{(10,70)} = 2.24$, and $F1_{(4,76)} = 2.96$, and * $F2_{(10,149)} = 2.15$, and $F2_{(4,155)} = 2.88$ at P = .025.

'' $F1_{(10,70)} = 2.59$, and $F1_{(4,76)} = 3.59$, and ** $F2_{(10,149)} = 2.46$, and $F2_{(4,155)} = 3.47$ at P = .01.

''' $F1_{(10,70)} = 3.48$, and $F1_{(4,76)} = 5.18$, and *** $F2_{(10,149)} = 3.24$, and $F2_{(4,155)} = 4.94$ at P = .001.

Ev.= expected vector and CV.= covariance matrix for the One Sample comparisons.

H Heterogeneous variances ($P \leq .05$).

P Mean response vectors are parallel.

Figure 5.8

Mean response vectors for comparisons (Table 5.12) of resident versus non-resident effects on larval preference for low conditioning.

A. 61PL(5)7+13 vs. 57PL(5)7+13

B. 61PL(5)7+13 vs. 59IL(1)7

C. 57PL(5)7+13 vs. 59IL(1)7

D. 62PL(2)13+13 vs. 58PL(2)13+13

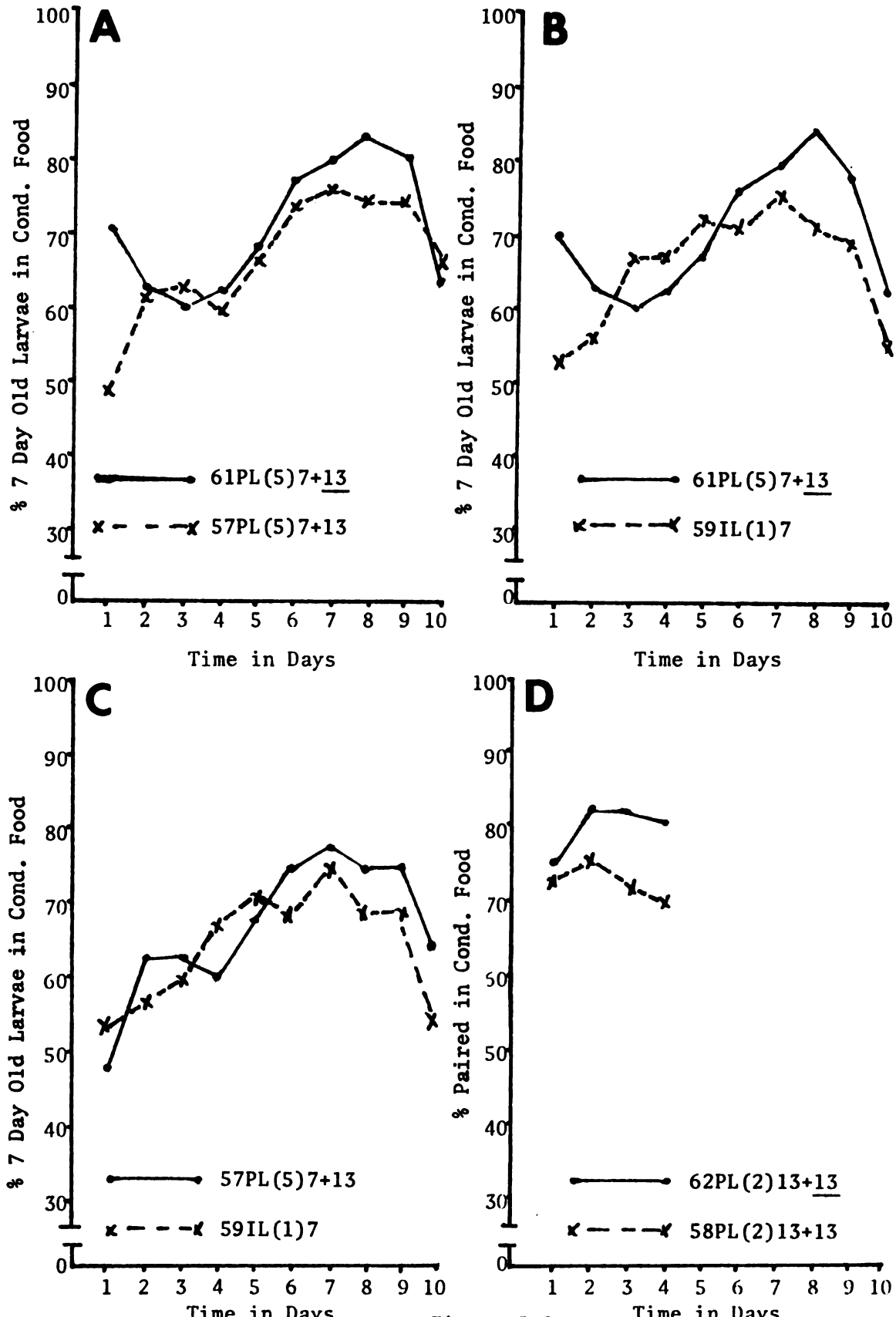

A
% 7 Day Old Larvae in Cond. Food
61PL(5)7+13
57PL(5)7+13
Time in Days
B
% 7 Day Old Larvae in Cond. Food
61PL(5)7+13
59IL(1)7
Time in Days
C
% 7 Day Old Larvae in Cond. Food
57PL(5)7+13
59IL(1)7
Time in Days
D
% Paired in Cond. Food
62PL(2)13+13
58PL(2)13+13
Time in Days

Figure 5.8

Figure 5.9

Mean response vectors for comparisons (Table 5.12) of resident versus non-resident effects on larval preference for high conditioning.

A. 68PH(5)7+13 vs. 64PH(5)7+13

B. 68PH(5)7+13 vs. 66IH(1)7

C. 64PH(5)7+13 vs. 66IH(1)7

D. 69PH(2)13+13 vs. 65PH(2)13+13

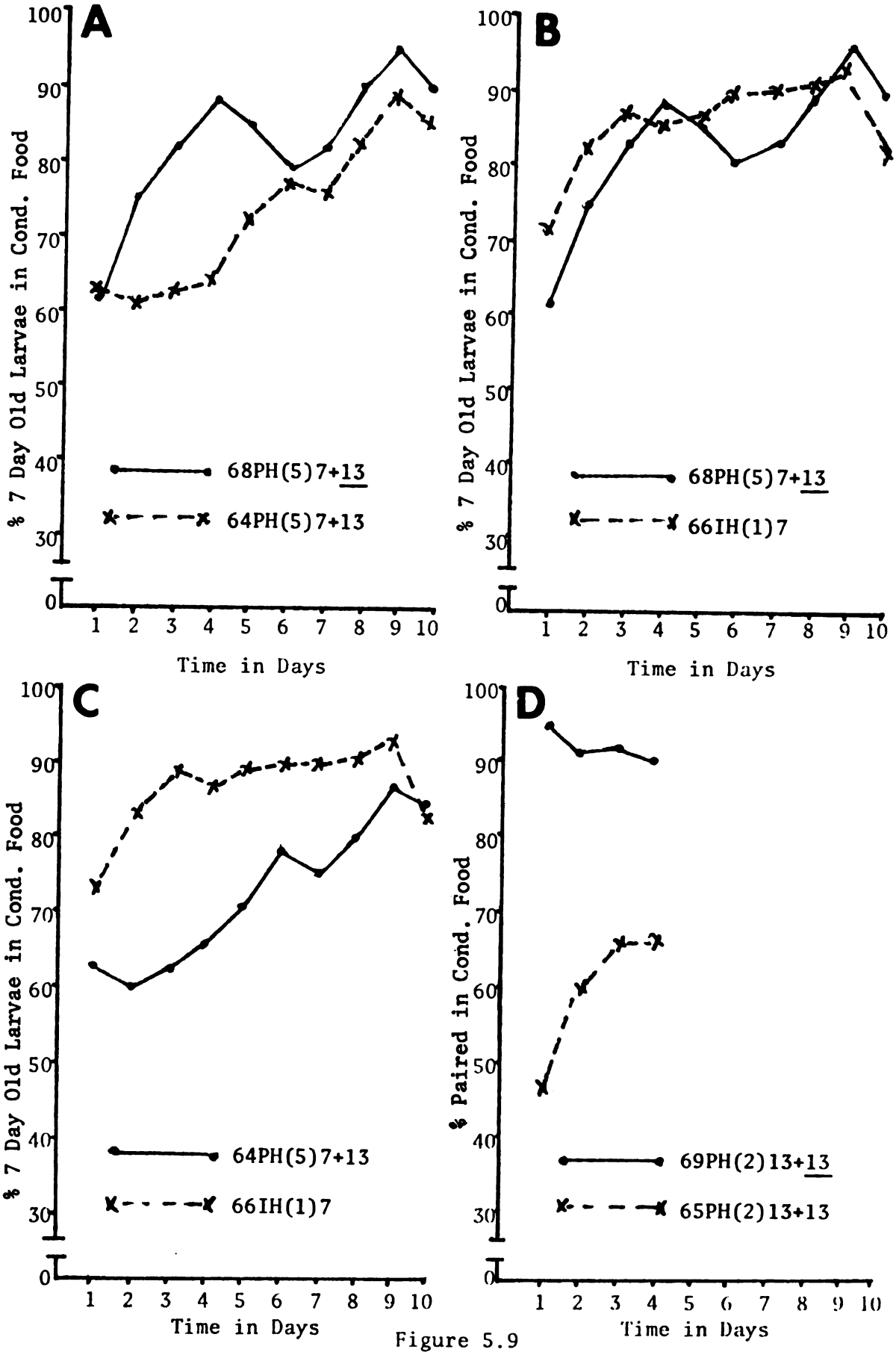

A
% 7 Day Old Larvae in Cond. Food
100
90
80
70
60
50
40
30
0
1 2 3 4 5 6 7 8 9 10
Time in Days
68PH(5)7+13
64PH(5)7+13
B
% 7 Day Old Larvae in Cond. Food
Time in Days
68PH(5)7+13
66IH(1)7
C
% 7 Day Old Larvae in Cond. Food
Time in Days
64PH(5)7+13
66IH(1)7
D
% Paired in Cond. Food
Time in Days
69PH(2)13+13
65PH(2)13+13

Figure 5.9

All larvae behave as if isolated. The One-Sample F1-values, however, indicate some differences possibly due to the difference in initial preferences as can be seen in Figure 5.8, although these differences are probably due to heterogeneous variances. The comparison 64PH(5)7+13 versus 66IH(1)7 is depicted for comparative purposes, as is comparison 64PH(5)7+13 versus 66IH(1)7. However, there are individual behavioral differences when conditioning is high, Table 5.12, and Figure 5.9. A 7 day old larva's preference for high conditioning is lowered when the 13 day old larva is introduced with it, but is not affected when the 13 day old larva is the resident in the conditioned food lump. The preference curve for the 7 day old larva when the resident is present is the same as the 7 day old isolate curve. This indicates that the significant animal-animal interaction is taking place when the 7 and 13 are initially placed in the center of the dish and not in the food lump. I will come back to this point in the discussion section. The two 13 day old larvae cannot be distinguished so the comparison was based on the number of pairs in conditioned food and it is noted that a 13 day old larva with a 13 day old resident is not affected but it is if both 13 day old larvae are introduced together.

In the next experiments I have selected only the situation where the 7 and 13 day old larvae are introduced together into a situation with high conditioning. These experiments will show that there is an interaction between biological conditioning and animal-animal interactions in the responses we have been examining.

Experiment III: Are the Lowered Responses of Younger, 7 Day Old Larvae, When a 13 Day Old Larva is Simultaneously Introduced, a Result of the Changing Biological Conditioning, Interaction with the 13 Day Old Larva, or Both?

In the last experiment we saw that a 7 day old larva was relatively unaffected by a 13 day old larva if the 13 day old larva was a resident in the highly conditioned food, but its preference for conditioned food was significantly lowered if the 13 day old larva was introduced at the same time. However, re-examination of the response vectors (see Table 5.12 and Figure 5.10) shows that the 7 day old response gradually rises to a level where it would have been if the 13 day old larva had not been present. In Figure 5.10, I have also graphed the response vector for the 13 day old larva including its wandering phase at which time it leaves the conditioned food and moves about the dish until a suitable pupation site is located and pupation occurs. Notice that as the 13 day old curve falls the 7 day old curve rises, the two crossing at about day 5 of the experiment, or when the 13 day old larva is 17 1/2 days old and the 7 day old is 11 1/2 days old. This suggests that when the 13 day old larva enters the wandering phase and vacates the conditioned food lump, the 7 day old larva moves to that lump, or that the 13 day old larva moves over to the 7 day old larva's lump, and in some way interacts with it causing the 7 day old larva to shift food lumps. There may, of course, be an interaction between the two. Since it is the older larva that seems to be determining the behavior of the 7 day old larva, older larva, i.e., 13 day old, will be used for manipulative purposes and only the 7 day old response will be monitored.

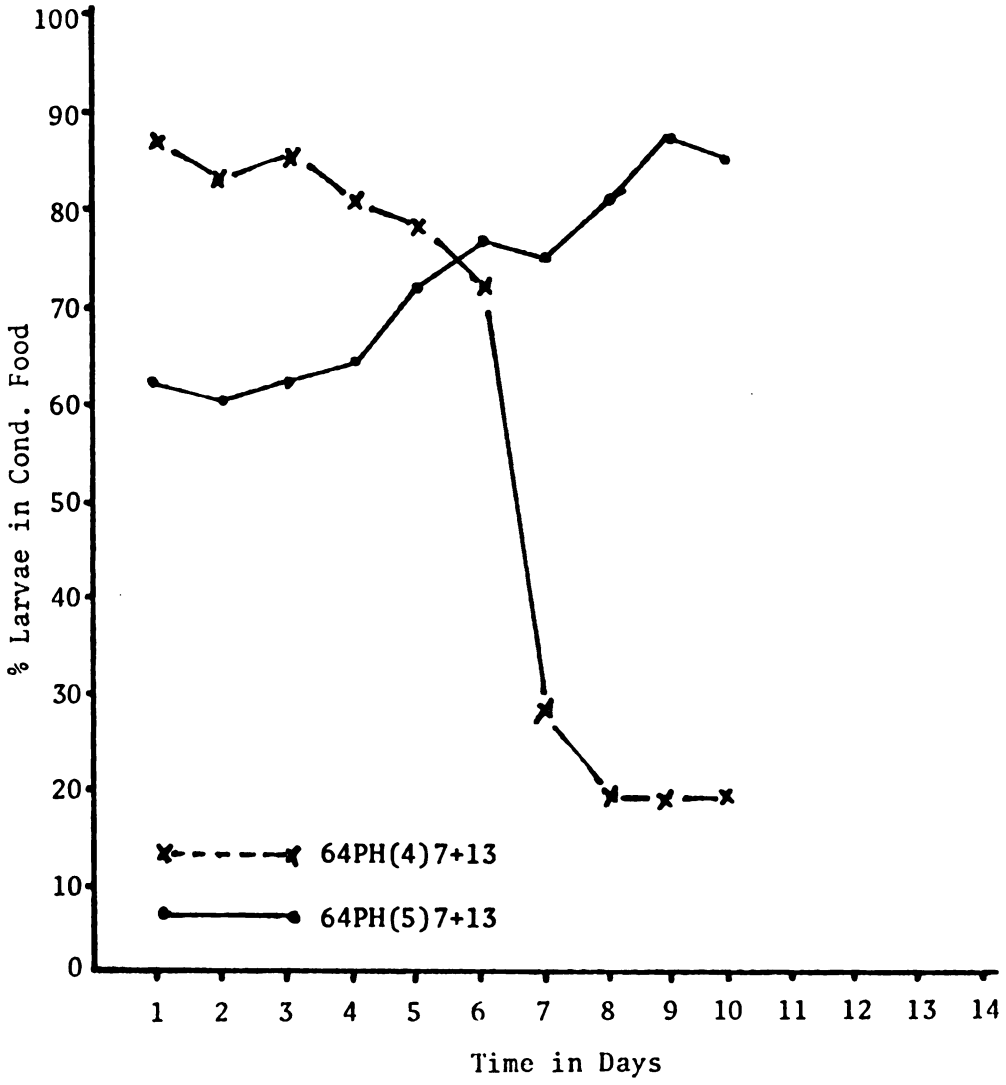


Figure 5.10

Mean response vectors of a 7 day old larva and a 13 day old larva with the wandering phase of the 13 day old larva included. These are from experimental group 64PH(1-5)7+13.

If the rise in the 7 day old response curve is a function of some interaction due to the wandering phase of the 13 day old larva, we should be able to shift the 7 day old curve two days earlier (to the left) or two days later (to the right) by pairing 7 day old larvae with 15 or 11 day old larvae, respectively. To test this hypothesis, the following 4-group experiment was designed. In one group, a 7 day old and a 15 day old larva were introduced, in another, a 7 and 13, and in a third a 7 and 11 were used. The fourth group is a 7 day old isolate larva with high conditioning. All experimental procedures are as previously described and one of the two food lumps was highly conditioned. For identification purposes the 4 groups are:

70PH(1-5)7+15

71PH(1-5)7+13

72PH(1-5)7+11

73IH(1)7

The mean response vectors ($\bar{X}$), estimated variance vectors (S^2), and D-values for each 7 and the 11, 13, and 15 day old larvae from these 4 groups are presented in Table 5.13 and graphed in Figure 5.11. However, only comparisons among the 7 day old response curves are of interest, but the response of the older larvae are shown for comparative purposes. Table 5.14 shows the results of the One- and Two-Sample comparisons among the three 7 day old preference curves when in the presence of high conditioning and either a 15, 13, or 11 day old conspecific.

It can be seen from the results of this experiment that the response to high conditioning of a 7 day old larva when paired with

Table 5.13

This table presents the response mean vectors($\bar{X}$), estimated variance vectors(S^2), and D-values for serial correlation for the groups to be analyzed in Table 5.14.

HIGH CONDITIONING

Group		Time in Days 1	2	3	4	5	6	7	8	9	10	D
70PH(5)7+15	$\bar{X}$	.587	.612	.725	.837	.912	.887	.900	.900	.925	.837	1.41*
	S^2	.245	.240	.202	.138	.081	.101	.091	.091	.070	.138	
71PH(5)7+13	$\bar{X}$	.600	.625	.587	.625	.725	.712	.825	.850	.875	.850	1.38*
	S^2	.243	.237	.245	.237	.202	.202	.146	.129	.111	.129	
72PH(5)7+11	$\bar{X}$	.625	.587	.600	.612	.675	.700	.687	.837	.900	.812	1.55*
	S^2	.237	.245	.243	.246	.222	.213	.218	.138	.091	.154	
73IH(1)7	$\bar{X}$	.712	.887	.875	.887	.925	.900	.900	.912	.900	.850	1.62*
	S^2	.207	.101	.111	.101	.070	.091	.091	.081	.091	.129	
70PH(4)7+15	$\bar{X}$	.850	.900	.912	.812	.800	.425					1.83I
	S^2	.129	.091	.081	.154	.162	.247					
71PH(4)7+13	$\bar{X}$	.850	.837	.850	.850	.787	.612	.337	.087			1.39*
	S^2	.129	.138	.129	.129	.169	.240	.226	.081			
72PH(4)7+11	$\bar{X}$	.837	.825	.862	.887	.837	.837	.875	.700	.250	.050	1.55*
	S^2	.138	.146	.120	.101	.138	.138	.111	.213	.190	.048	

*$D_{L(6,80)} = 1.83$ and $D_{U(6,80)} = 2.16$ at P = .05.

*$D_{L(8,80)} = 1.77$ and $D_{U(8,80)} = 2.22$ at P = .05.

*$D_{L(10,80)} = 1.71$ and $D_{U(10,80)} = 2.28$ at P = .05.

I D-value was inconclusive.

Figure 5.11

Mean response vectors of 7 day old larvae to high conditioning when a 15, 13, or 11 day old conspecific is also present. The mean response vectors for the 15, 13, and 11 day old larvae are shown, with their wandering phases, for comparative purposes.

Illustrated are the mean response vectors for:

A. 7 and 15 day old larvae.

B. 7 and 13 day old larvae.

C. 7 and 11 day old larvae.

D. All the 7 day old response vectors from A, B, and C, as well as the response vector for a 7 day old isolate to the same initial degree of conditioning.

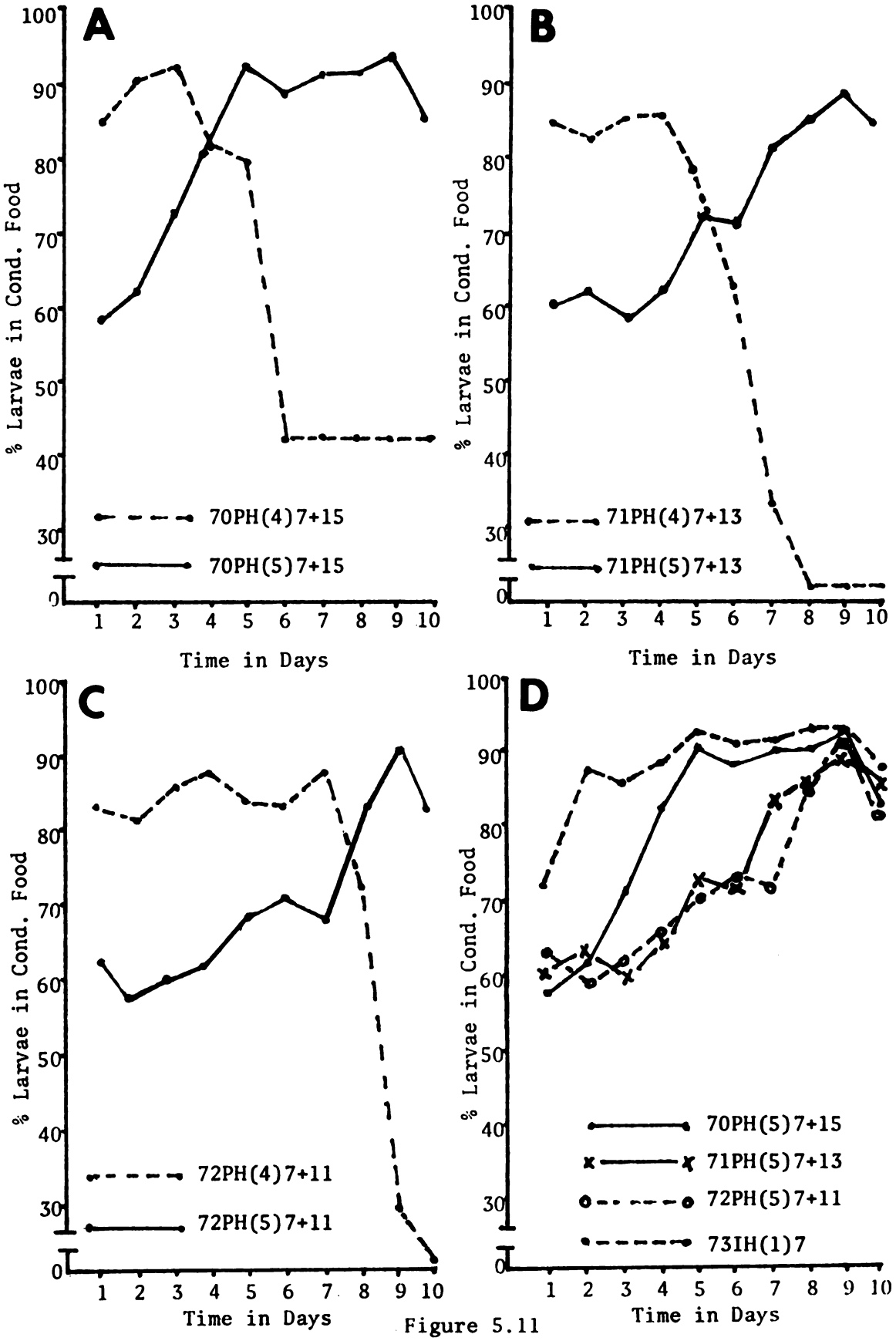

A
B
C
D
% Larvae in Cond. Food
Time in Days
70PH(4)7+15
70PH(5)7+15
71PH(4)7+13
71PH(5)7+13
72PH(4)7+11
72PH(5)7+11
70PH(5)7+15
71PH(5)7+13
72PH(5)7+11
73IH(1)7

Figure 5.11

Table 5.14

Hotelling's One(') and Two(*) Sample comparisons of the 7 day old mean response vectors when paired with an 11, 13, or 15 day old larva or when an isolate, all with initial high conditioning on one food lump.

Comparisons EV.	CV.	F1	F2	Box's Test	T^2 Test for Parallelism
71PH(5)7+13 vs.	70PH(5)7+15		2.24*	H	
71PH(5)7+13 vs.	72PH(5)7+11	2.16	0.90	H	P
72PH(5)7+11 vs.	70PH(5)7+15		2.87*	H	
73IH(1)7 vs.	70PH(5)7+15	7.08'''	1.96	H	
73IH(1)7 vs.	71PH(5)7+13		2.60*	H	
73IH(1)7 vs.	72PH(5)7+11		3.19*	H	

$^{'}F1_{(10,70)} = 2.24$ and $^{*}F2_{(10,149)} = 2.15$ at P = .05.

$^{''}F1_{(10,70)} = 2.59$ and $^{**}F2_{(10,149)} = 3.59$ at P - .01.

$^{'''}F1_{(10,70)} = 3.48$ and $^{***}F2_{(10,149)} = 5.18$ at P = .001.

EV.= expected vector and CV.= covariance matrix for the One Sample comparisons.

^{H}Heterogeneous variances ($P \leq .05$).

^{P}Mean response vectors are parallel ($P \leq .05$).

a 15 day old larva is significantly higher than its response when paired with a 13 or an 11 day old larva. However, the 7 day old response when paired with a 13 day old larva is not different from when paired with an 11 day old larva. In Figure 5.11, it can be seen that as either the 15 or 13 day old larvae enter the wandering phase, their response curves begin to fall and that of the 7 day old response to conditioning begins to increase. In the 7+11 situation, however, the 7 day old response is on the rise before the 11 day old larva enters the wandering phase. It can be seen also that the response of a 7 day old larva to high conditioning is significantly lower when an 11 or 13 day old larva is present than when the 7 day old larva is alone. The 7 day old response when with a 15 day old larva, however, is not different from the isolated condition, although it would be at the .05 level, and is different with the One-Sample test, indicating a problem due to heterogeneous variances.

All comparisons, except one, yield non-parallel responses indicating the possibility that the 7 day old larvae are utilizing different behaviors in their response when a conspecific is present, than when isolated. These responses also appear to be different with different aged conspecifics, except in the 7+11 versus 7+13 situation.

It is very tempting to say that the response of 7 day old larvae to conditioning is lower because it has been repelled by the older larva. However, in Table 5.15 are presented the results of comparing the first three days of each 7 day old response mean vector to a vector whose entries are all 0.5 to see if repulsion has occurred. These results show (in combination with Figure 5.11) that the initial

Table 5.15

Hotelling's One Sample (') comparison of the first 3 days of the 7 day old response vectors when with various aged conspecifics and when isolated, with an expected vector whose entries are all 0.5.

Comparisons	F1
70PH(5)7+15	7.12"
71PH(5)7+13	1.74
72PH(5)7+11	1.95
73IH(1)7	43.15"

'$F_{(3,77)}$ = 3.29 at P = .025.

"$F_{(3,77)}$ = 6.02 at P = .001.

responses, although lower than if isolated, are all either not different from random or significantly higher than random. Therefore, although the presence of an older conspecific significantly lowers the response of a 7 day old larva to conditioning, the term repulsion is not appropriate since the responses are not significantly lower than random.

These results are suggestive that the response of a 7 day old larva to conditioning when paired with an older conspecific may be the result of an interaction between biological conditioning and animal-animal interaction. The initial lower response in each case being due to some interaction between the 7 day old larva and the conspecific in the center of the dish before either larva has sampled the food lumps. The rise in the 7 day old response with time, however, is more difficult to explain. Except when paired with an 11 day old conspecific, the 7 day old response seems to coincide with the 13 or 15

day old wandering phase. It may be that when the older larva leaves the conditioned lump it goes over to the 7 day old's lump and a second interaction occurs causing the 7 day old larva to move to a new food lump. However, it must be remembered that when the older larva chooses the originally conditioned lump, it spends at least a day, and in the case of the 13 day old larva, 3 days, adding its own conditioning to that already present. Therefore, when the older larva leaves this lump, the 7 day old larva may be responding to the extra conditioning. There may also be an interaction between these two events. The following experiment was designed to look at these possibilities, using only the 7+13 situation since the greatest effects on the 7 have been with a 13 day old larva.

The next hypothesis to be examined is that the response of the 7 day old larva to conditioning when a 13 day old larva is present is due to an interaction between three variables: (1) the initial encounter between the two larvae in the middle of the dishes, (2) the extra conditioning that the 13 day old larva adds to the already conditioned lump, and (3) a second interaction between the two larvae when the 13 begins wandering. These events will henceforth be referred to as 1, C, or 2 respectively. The following experimental groups were set up with various combinations of the above variables:

74IH(1)7(0-0-0) : This is the 7 isolate situation having none of the above variables operating.

75PH(5)7+13(1-C-2): This is the situation in which a 7 and 13 day old larva are placed in the experimental situation and the 7 is monitored for the total experimental period.

76PH(5)7+13(1-0-0): In this group the 7 and 13 day old larvae are placed in the experimental situation and in 12 hours the 13 is removed. Therefore, the 7 received the initial encounter but not the second encounter and there was no extra conditioning being done by the 13, or at least only a very small amount in 12 hours.

77PH(5)7+13(1-C-0): The 7 and 13 were placed in the apparatus and when the 13 day old larva was 16 days old, just before wandering, it was removed from the dish. Thus, the 7 received the initial encounter and the extra conditioning, but not the second encounter with the 13 day old larva.

78PH(5)7+13(1-0-2): The 7 and 13 were placed in the dishes and 12 hours later the 13 was removed. Four days later, or when the 13 would have begun wandering, a wandering larva from the colony was placed in the dishes. Therefore, the 7 day old larva received the initial encounter and second encounter but no extra conditioning had been done by the 13 day old larva.

Each of these 5 groups received different manipulations throughout the experimental period, and as a control for this the following was done. Whenever the 13 day old larva was removed from the dishes in the respective groups, the dishes in the groups not receiving this treatment were opened and the food lumps gently tapped with a pair of forceps. When a wandering larva was added to group 78PH(5)7+13(1-0-2) all other dishes were opened and the center floor of the dish gently tapped with forceps.

The mean vectors ($\bar{X}$), estimated variance vectors (S^2) and D-values for serial correlation are shown in Table 5.16 for the 7 day old test larvae in each group. The results of Hotelling's One- and Two-Sample comparisons between all possible combinations of 7 day response vectors from Table 5.16 are shown in Table 5.17. The

Table 5.16

Table of mean response vectors($\bar{X}$), estimated variance vectors(S^2), and D-values for serial correlation of the 7 day old larvae from the experimental groups being tested for the effects of initial encounter, extra conditioning, and the second encounter with a 13 day old conspecific.

Groups		Time in DAys 1	2	3	4	5	6	7	8	9	10	D
74IH(1)7(0-0-0)	$\bar{X}$	.725	.837	.862	.887	.925	.887	.900	.925	.925	.850	1.33*
	S^2	.202	.138	.120	.101	.070	.101	.091	.070	.070	.129	
75PH(5)7+13(1-C-2)	$\bar{X}$	.600	.600	.637	.625	.712	.750	.762	.825	.875	.825	1.19*
	S^2	.243	.243	.243	.237	.207	.190	.183	.146	.111	.092	
78PH(5)7+13(1-0-2)	$\bar{X}$	.625	.575	.612	.612	.737	.787	.800	.787	.762	.725	1.32*
	S^2	.237	.247	.240	.240	.196	.169	.162	.169	.183	.202	
77PH(5)7+13(1-C-0)	$\bar{X}$	.562	.587	.600	.612	.687	.712	.700	.737	.725	.700	1.35*
	S^2	.249	.245	.243	.240	.218	.207	.213	.196	.202	.213	
76PH(5)7+13(1-0-0)	$\bar{X}$	.587	.587	.587	.612	.625	.637	.637	.637	.650	.587	1.04*
	S^2	.245	.245	.245	.240	.237	.234	.234	.234	.230	.245	

*$D_{L(10,80)} = 1.71$ and $D_{U(10,80)} = 2.28$ at $P = .05$.

Table 5.17

Results of Hotelling's One(') and Two(*) Sample comparisons among the 7 day old response vectors in Table 5.16.

Comparisons EV.	CV.	F1	F2	Box's Test	T^2 Test for Parallelism
(1-C-2) vs.	(0-0-0)		2.17*	H	P
(1-0-2) vs.	(0-0-0)		2.93**	H	P
(1-C-0) vs.	(0-0-0)		3.28**	H	P
(1-0-0) vs.	(0-0-0)		3.73***	H	P
(1-0-2) vs.	(1-C-2)	2.31'	0.85	H	P
(1-C-0) vs.	(1-C-2)	2.19	0.95	H	P
(1-0-0) vs.	(1-C-2)	4.35'''	1.58	H	P
(1-C-0) vs.	(1-0-2)	0.97	0.44	H	P
(1-0-0) vs.	(1-0-2)	2.11	0.99	H	P
(1-0-0) vs.	(1-C-0)	0.68	0.35	H	P

' $F1_{(10,70)} = 2.24$ and $^{*}F2_{(10,149)} = 2.15$ at P = .025.

'' $F1_{(10,70)} = 2.59$ and $^{**}F2_{(10,149)} = 2.46$ at P = .01.

''' $F1_{(10,70)} = 3.48$ and $^{***}F2_{(10,149)} = 3.24$ at P = .001.

EV.= expected vector and CV.= covariance matrix for the One Sample Test.

^{H}Heterogeneous variances ($P \leq .05$).

^{P}Mean response vectors are parallel ($P \leq .05$).

response mean vectors for the 7 day old larvae from all groups are graphed in Figure 5.12. (For the purposes of identifying these groups in the tables and figures, I have used only the three numbers for the variables in question, i.e., 1-C-2, 1-C-0, 1-0-0, 1-0-2, or 0-0-0. Since all groups deal with only the response of the 7 day old larva this system makes it clearer which variables are being referred to.) It can be seen from the Two-Sample comparisons (Table 5.17) that the 7 day old isolate response to conditioning is significantly higher than all the others, but that there are no other significant differences. The One-Sample test elucidates only two other differences. All responses are parallel, however, indicating similar behaviors but at different levels.

These results were a bit surprising in light of the separation of response curves in Figure 5.12. However, examination of the data indicates some possible reasons for the results obtained. The range in which the responses have in which to separate themselves out is a relatively narrow one during the time in which the responses begin to exhibit differences; from about 60% to 85%. The variances are also heterogeneous, as attested to by Box's test and the results of the One-Sample test.

Throughout this chapter, as well as Chapter IV, I have alluded to this fact. If we examine the variance vectors for these groups, and in fact for all groups in this Chapter, an interesting trend will be noted. As response to conditioning increases the variances decrease. Therefore, the variance vectors may be a good mirror for larval activity. For example, examine the variance vectors for groups

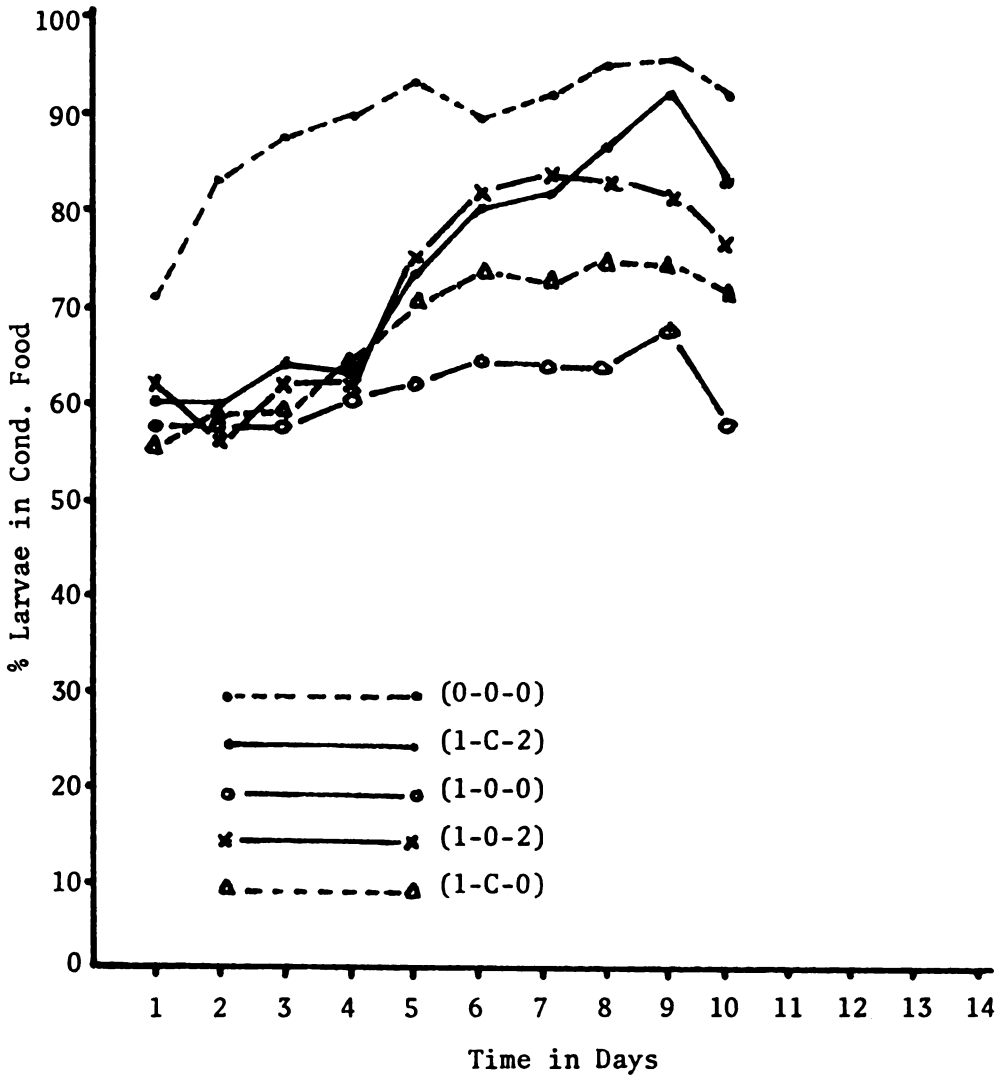


Figure 5.12

Response mean vectors for the 7 day old larvae from all experimental groups in Tables 5.16 and 5.17.

1-C-2 and 1-0-0 and notice that in Figure 5.2, as the response vector for 1-C-2 rises, the variances get smaller, perhaps indicating a more stable activity of the larva as they select and stay in the conditioned food. The variances for group 1-0-0, however, remain large, and as can be seen, the response vector for this group never rises much. That is, this group does not get to conditioned food and their activity is high or fluctuating. This is the most probable explanation for the lack of significant results in the comparisons in Table 5.17.

Because of the heterogeneous variances, it was decided to analyze the last three data points for each of the response vectors. It can be seen from Table 5.16 and Figure 5.12 that the variances have stabilized, although still large in some groups, and the response vectors have relatively stabilized at this time. We would not expect to get some of the same differences in this comparison since some of the responses are now at the same level. For example, groups 1-C-2 and 0-0-0 are no longer different because 1-C-2 is at the same level as 0-0-0 in the latter stages of the experiment. Table 5.18 shows the results of these comparisons. The only new difference now detected is that 1-0-0 is a lower response than 1-C-2. Tables 5.17 and 5.18, therefore, indicate the following.

All manipulations produced significantly lower responses on the part of the 7 day old larvae than for the response of isolated 7 day old larvae. That is, the presence of an older larva, even if only for 12 hours, is a sufficient stimulus to prevent the response of 7 day old larvae to conditioning from reaching the level of an isolate's response. This is also a sufficient stimulus for preventing 7 day

Table 5.18

Results of Hotelling's One(') and Two(") Sample comparisons among the 7 day old response vectors in Table 5.16 for the last 3 days of each mean response vector.

Comparisons EV.	CV.	F1	F2	Box's Test	T^2 Test for Parallelism
(1-C-2) vs.	(0-0-0)	4.96''	1.58	H	P
(1-0-2) vs.	(0-0-0)	10.11'''	2.81	H	
(1-C-0) vs.	(0-0-0)		3.95*	H	P
(1-0-0) vs.	(0-0-0)		7.66***	H	P
(1-0-2) vs.	(1-C-2)	5.69''	2.01	H	P
(1-C-0) vs.	(1-C-2)	6.87'''	2.77	H	P
(1-0-0) vs.	(1-C-2)		4.40**	H	P
(1-0-0) vs.	(1-C-0)		0.94		P
(1-0-2) vs.	(1-0-0)	3.84'	1.67	H	P
(1-C-0) vs.	(1-0-2)		0.20		P

' $F1_{(3,77)} = 3.29$ and * $F2_{(3,162)} = 3.22$ at P = .025.

'' $F1_{(3,77)} = 4.05$ and ** $F2_{(3,162)} = 3.94$ at P = .01.

''' $F1_{(3,77)} = 6.01$ and *** $F2_{(3,162)} = 5.78$ at P = .001.

EV.= expected vector and CV.= covariance matrix for the One Sample Test.

H Heterogeneous variances ($P \leq .05$).

P Mean vectors are parallel ($p \leq .05$).

old larvae from reaching the same response level as a 7 day old larva that received the two encounters with the older larva and the extra conditioning. The other two groups are intermediate. That is, the groups that received both encounters but no extra conditioning or only the first encounter with extra conditioning are statistically not different from the group that received only the first encounter or the group receiving all three stimuli. However, the trend for the response vectors of these two groups is that they lie between the 1-0-0 and 1-C-2 groups. It would also appear that the 1-C-0 group is closer to the 1-C-2 group. This may indicate that the extra conditioning is not as necessary to overcome the initial encounter (i.e., 1-C-0) as is the second encounter without the conditioning (i.e., 1-0-2). The F2-values also indicate this trend. Although suggestive, these "trends" are not significantly different from one another. It would also appear that these experiments have not yet made it possible to make definitive statements about certain aspects of the interaction between biological conditioning and animal-animal interactions, although there are some strong indications. This will be discussed in the discussion section.

A point made earlier in this chapter is worth repeating at this time. Even though the 7 day old response vectors are significantly lowered by the presence of an older conspecific, they are not being repulsed. If repulsion were the mechanism, then we would expect the response vectors to be significantly below random, i.e., below 50%. Table 5.19 shows the results of comparing the first 5 days of response for each experimental group, since it is during the

Table 5.19

Results of Hotelling's One Sample comparison of the first 5 data points of each group from Table 5.16 with an expected vector whose entries are all 0.5.

Group	F1
0-0-0	277.62"'
1-C-2	4.22"
1-0-2	131.42"'
1-C-0	2.78'
1-0-0	1.31

'$F_{(5,75)}$ = 2.74 at P = .025.

"$F_{(5,75)}$ = 3.28 at P = .01.

"'$F_{(5,75)}$ = 4.64 at P = .001.

first half of the experimental period that all responses are lowest. The results show that all groups are either random or significantly above random.

These differences would not be as great if fewer data points had been used, but the point is that all groups, except group 1-0-0, are significantly different from random in the direction of attraction to conditioned food and therefore not repulsed in the strict sense of the word. Group 1-0-0 is also not repulsed but is randomly responding. This may indicate the need for a second stimulus to counteract the effect of the initial encounter with the 13 day old larva.

Throughout this chapter, several groups have been replicated for various experiments. In particular, the 7 day old isolate group

and the 7 day old group when paired with a 13 day old larva have each been replicated three times. Table 5.20 presents the results of comparing these replicates to see if they differ from one experiment to the next.

Table 5.20 demonstrates that all comparisons are not significantly different with the Two-Sample test, but that one is different with the One-Sample test. This is the first hint that populations of larvae may be genetically changing with time.

Discussion

Invertebrate studies indicate that spatial distribution is a function of an interaction between biological conditioning and animal-animal interactions. These studies, however, are population studies in which it is impossible to separate individual behavior from group behavior, and there has been no systematic attempt to do so. In Park's work (1934, 1935) the distribution of Tribolium confusum was concluded to be heavily dependent on the amount of homotypic conditioning. However, as population size increases, not only does conditioning increase, but the number of adults and larvae increases. What appear to be responses to increased conditioning may well be responses to increased density or an interaction between the two. Naylor (1959) attempted to separate these variables and was partially successful. His results indicate that spatial patterns in adult Tribolium are primarily dependent upon interactions between adults as density increases but that these patterns are modifiable when sexual relationships and biological conditioning are introduced as variables. However, Naylor was not able to determine the relationship between

Table 5.20

Results of Hotelling's One(') and Two(*) Sample comparisons of various groups replicated throughout this Chapter.

Comparisons EV.	CV.	F1	F2	Box's Test	T^2 Test for Parallelism
73IH(1)7	vs. 66IH(1)7	2.35'	0.82	H	P
73IH(1)7	vs. 74IH(1)7+13(0-0-0)	0.72	0.30	H	P
66IH(1)7	vs. 74IH(1)7+13(0-0-0)	1.71	0.67	H	P
75PH(5)7+13(1-C-2)	vs. 64PH(5)7+13	0.39	0.17	H	P
75PH(5)7+13(1-C-2)	vs. 71PH(5)7+13	1.24	0.52	H	P
71PH(5)7+13	vs. 64PH(5)7+13	1.26	0.61	H	P

' $F1_{(10,70)} = 2.24$ and * $F2_{(10,149)} = 2.15$ at P = .025.

'' $F1_{(10,70)} = 2.59$ and ** $F2_{(10,149)} = 2.46$ at P = .01.

EV.= expected vector and CV.= covariance matrix for the One Sample Test.

H Heterogeneous variances ($P \leq .05$).

P Mean response vectors are parallel ($P \leq .05$).

biological conditioning and adult interactions partly because he did not utilize a graded series of conditionings. Wellington's (1957) work with tent caterpillars was free of the adult-young interactions of Park's (1935) work, but he did not determine whether the preferences of these larvae for homotypic conditioning was affected by the presence of conspecifics. In 1963a and 1964e, Surtees concluded from his studies on grain weevils that spatial patterns in high densities of weevils are a result of changes in individual behavior as a result of stimulation from conspecifics. However, his studies are confounded by the presence of high biological conditioning which he neither considered nor controlled for.

A previous paper (Chapter IV) demonstrated that the distribution of isolate larvae is a function of larval conditioning and that preference for conditioned medium is a function of the degree of conditioning and the age of the responding larva. The rationale for my studies in this chapter has been that the preference of individual Galleria larvae for homotypic conditioning is a function of the degree of conditioning, the presence of a conspecific, and the age and location of the conspecific.

Individual Responses to a Conspecific and Biological Conditioning

When there is no previously conditioned food available, larvae tend to distribute themselves randomly when isolated or when a conspecific is present. There is, initially, neither an attraction or a repulsion between pairs of larvae regardless of age. With time, however, larvae begin to aggregate, apparently as a result of their

own conditioning and possibly due to the development of interactions between different aged larvae. It was demonstrated, for example, that if one member of a pair of larvae is a 13 day old larva, aggregation does not occur and larval distribution remains random. Younger larvae do begin to aggregate and the most significant case was the 7 and 11 day old situation. Analysis further demonstrated that the 7 day old larva is moving to the food lump on which the 11 day old larva is residing, probably as a result of the 11 day old larval conditioning being greater. However, such aggregation behavior would also necessitate that each larva tolerate the close proximity of another larva, whereas this is not the case with a 13 day old organism. The lack of aggregation with 13 day old larvae is probably not because 13 day old larvae are secreting a qualitatively different conditioning than younger larvae, since isolate 7 or 11 day old larvae have been shown to be attracted to the same degree of conditioning when the 13 day old larva is absent (see Chapter IV).

Situations with two 7 or two 11 day old larvae also exhibit aggregation behavior. This is probably not a case of one larva's conditioning being greater than the other and thus attracting the other larva, although there may be individual differences in conditioning behavior. Younger larvae do not initially respond to each other, but when they each begin conditioning and connecting the food lumps with silk tunnels this may increase the probability of finding them in pairs. This behavior may also be related to activity rhythms. Long (1955) demonstrated that different ages of various butterfly larvae exhibit different activity rhythms. He found that the closer

to pupation these larvae get, the more "restless" they become. This restlessness does not necessarily mean they move about more, only that they are more easily agitated and exhibit rather sudden and jerky movements when touched. Younger larvae tend to be more docile and move about with more easy-going motions. Therefore, young larvae may tolerate the presence of young, or not very old larvae, since their activity rhythms are more synchronous whereas the activity patterns of older larvae are disruptive to group formation.

Experiment II indicates that larval interactions are associated with the presence of an already conditioned food lump. The preference behavior of individual larvae to low-conditioned food is the same whether isolated or with a conspecific, regardless of the age of the conspecific. As seen in Chapter IV, however, the response of isolate larvae to this degree of conditioning, particularly for a 7 day old larva, is already at a relatively low level and it would almost require repulsive behavior to detect a difference between the isolate and group situation. When one food lump is highly conditioned, however, the preference of individual larvae is affected by the presence of a conspecific, regardless of the age of the conspecific. For example, the total number of 7 day old larvae on conditioned food when two 7 day old larvae are present is lower than when only one larva is present. This distinction is difficult to elucidate since the two larvae are not individually distinguishable. However, it can be definitively seen that a 13 day old larva's response to conditioning is lowered when a 7 day old larva is present and a 7 day old's response is lowered when a 13 is present, as compared to the isolate

situation. This suggests that although the greatest effect is on the 7 day old larva's response, the 7 day old larva also affects the response of an older larva.

The response of individual larvae to a conditioned food lump of low or high conditioning is unaffected by the presence of a resident larva (13 day old larva) in that lump. That is, individuals respond to such a situation as if the resident were not present. The conditioning may act as a buffer, allowing organisms to be paired that do not pair in the absence of conditioning. It is also possible that the test larva and the resident never encounter one another in the conditioned lump.

Experiment III elucidated the results of Experiment II, in that 7 day old response to highly conditioned food is lowered when a 15, 13, or 11 day old larva is introduced with the 7. There may be a relationship between the wandering phase of the older larva and the rise in response of the younger larvae. The 7 day old response begins increasing two days earlier when a 15 day old larva is present than when a 13 day old larva is present, the wandering phase of a 15 day old larva occurring two days sooner than for a 13 day old larva. However, the response vector of the 7 day old larva begins rising earlier than predicted when an 11 day old larva is present. This may be a function of the initial encounter with an 11 day old larva not being as traumatic for a 7 day old larva as it is with a 13 or 15 day old larva. This result also supports the finding in Experiment I that 7 day old larvae are attracted to where the 11 day old larva is residing.

Further experiments attempted to dissect the behavior of the younger larva with the hypothesis that its lower response was due to an interaction between the initial encounter with the older larva, the extra conditioning the older larva adds to the already conditioned food lump, and a second encounter when the older larva begins wandering. The results demonstrated that any combination of these variables is sufficient to significantly lower the younger larva's response from that of the isolate situation. It was also found that the situation in which only the initial encounter with the older larva was the variable that response was significantly lower than when all three variables were present. No other differences were found, although there is a decided trend for the situations in which both encounters are present or only the first encounter and extra conditioning were present, to be intermediate between the others. There are several reasons why these trends were not significant and they will be discussed in the next section.

The general conclusion from all of these studies is that individual behavior and distribution are functions of the degree of homotypic conditioning, age of individual larvae, presence of a conspecific, and location of the conspecific. There are some indications from these studies that differences in larval activity may play a role in spatial distribution. By larval activity I am referring to the time in development when larvae tend to move from one food lump to another, but not movement within any one food lump. For example, it was demonstrated in Chapter IV that 7 day old larvae are less active during the first 2-3 days of the experiment, as measured by the number of times

they move from one food lump to another, whereas 13 day old larvae are more active at that time. Mobility differences, on the other hand, refer to the ability of larvae to move from one food lump to another. It was previously pointed out (Chapter IV) that older larvae may be more mobile than younger larvae and can more easily cross the open space between food lumps.

In the next section I will discuss the possibility that differences in larval activity may play a role in the spatial distribution of Galleria larvae, and propose several hypotheses for future testing. The discussion, however, will be mostly theoretical.

Possible Mechanisms for the Interaction of Biological Conditioning and Larval-Larval Interaction in the Spatial Distribution of Galleria Larvae

In all of the experiments presented in this chapter, the only situations in which larval response to conditioning was significantly lower than in the isolate situation were when larvae initially encountered each other in the center of the experimental dishes and when a highly conditioned food lump was present at the start of the experiment. Assuming high conditioning to be a preferred stimulus, some sort of competition may be involved in the larval interactions for it. However, we saw in Chapter IV, that older isolate larvae exhibit a higher initial preference for highly conditioned food than do younger larvae, and it was postulated that this is related to activity differences between the larvae. Older larvae find the conditioned food lump sooner than young larvae and cease their movements in the preferred stimulus, although differential sensory capabilities

may also be involved. The young larvae, on the other hand, tend to remain in the first food lump encountered for a period of time and move to the conditioned food lump later in development. If now, a younger larva encounters an older larva in the center of the dish and if the older larva is preferentially heading for the conditioned food, the younger larva may merely head in the opposite direction as an "escape" reaction, which would enhance the normally lower initial response of young larvae. I have also observed that these larvae release some sort of liquid substance if disturbed. Upon encountering each other, in the center of the dish this substance may be released, causing avoidance between the two larvae and differentially having a greater effect on the younger larva. It is not clear what constitutes the interaction, but it is clear from these experiments that the initial interaction is a prerequisite for lowered responses to conditioned food. It is also indicated that this interaction is less traumatic when between two larvae of not very different ages. For example, the 7 day old larva's response curve rises relatively sooner when the interaction was between a 7 and 11 day old larva than between a 7 and 13 or 15 day old larva.

When the 7 day old response curve begins to exhibit a rise, I have postulated that it is a result of a second interaction between the two larvae, or a response to the extra conditioning the older larva has done to the already conditioned food, or an interaction between the two. These variables may be linked to activity differences. After the initial encounter between the two larvae, the 7 day old response to conditioning is depressed and remains so for about four

days. At the end of those four days the 13 day old larva is entering the wandering phase and leaves the conditioned lump. At the same time, the 7 day old larva, which is now 11 days old, may be more active and begins to explore its environment. This was indicated in Chapter IV. It thus encounters the highly conditioned food which is more highly conditioned than the food it just left and it remains there. Thus its response to conditioning increases. This would explain why the 7 day old response increases sooner when an 11 day old larva is present but which is not yet wandering. If this were the only mechanism, however, we would predict that the response curve for the group in which only an initial encounter with the 13 day old larva took place, to begin an upswing about four days into the experiment and reach the same level as the situation in which both encounters and extra conditioning were present. Experiment III, however, demonstrated that those two situations remain significantly different. Of course, in the situation where only the initial encounter occurred, the highly conditioned food lump did not receive any extra conditioning. However, if the 7 day old larva does enter a more active phase, it should have found that lump and remained there since it was still more highly conditioned than its own lump. Some other mechanism(s) must be operating.

It was noted earlier that the variances for the response of larvae to conditioning, whether paired or isolates, decrease as response to conditioning increases. This may indicate that less movement between food lumps occurs as larvae find and stay in the conditioned food. This is particularly obvious for the situation where

a 7 day old isolate has a choice between a plain and a conditioned food lump. As more and more larvae find the conditioned lump, they remain there and activity, as measured by exchanging food lumps, ceases. In the situation where a 13 day old larva was introduced with the 7 day old larva, we find that about 60% of the 7 day old larvae initially choose the conditioned and 40% the plain lump. However, perhaps there is movement between the two lumps. That is, there may be a constant exchange between the two food lumps. This would not be the case if the 7 had been isolated for the whole experiment and the hypothesis is that the initial encounter with the 13 day old larva may have in some way altered the activity of the 7 day old larva and that a second stimulus, i.e., extra conditioning, a second encounter with the 13, or both is needed to drive the 7 day old response up to the level where it would have been if it had been an isolate to begin with. Long's (1955) data tends to support this postulation. He demonstrated that young larvae are relatively less active than older larvae of the large white butterfly and that older larvae will disrupt the activity rhythm of a young feeding group.

Based on the foregoing considerations the following system is being postulated for future testing:

1. Young larvae are initially less active, as measured by exchanging food lumps, than older larvae but their activity increases as they age. This was demonstrated in Chapter IV and is also evident from my data sheets with young isolate larvae in a situation with two initially plain food lumps. The response in such a situation is always random over time,

but about 5 days into the experiment, many of these isolates switch food lumps and remain there.

2. High degrees of conditioning are a preferred stimulus and when encountered activity decreases. This was also earlier demonstrated and is supported by examining the variances for such situations. The variances decrease as highly conditioned food is encountered.
3. Older larvae, being more active, have two effects on younger larvae. They depress the younger larva's response to highly conditioned food and possibly alter its activity in such a manner that young larvae tend to remain in the initially chosen food lump.
4. Young larvae, which have been so affected by an older larva, may require a second stimulus to offset their tendency to remain in the initially chosen non-conditioned food lump and to find and settle in the conditioned food when the older larva is gone. Such a second stimulus may be in the nature of a second encounter with the older larva or the presence of extra conditioning.

There may be an inherent activity rhythm in these larvae, in which, as they age, they become investigatory and begin sampling their environment and moving between food lumps. If a highly conditioned food lump is encountered they may cease this activity and settle down. If they had been in such a conditioned food lump initially they may sample the plain lump but return to the preferred stimulus. If young larvae initially encounter an older larva in the open, i.e., not in a

conditioned lump, the effect is to alter the young larva's behavior so that its preference for conditioned food is lowered and its activity is affected in such a way that normally conditioned food is not a sufficient stimulus to offset it. Therefore, many exchanges might occur between plain and conditioned lumps. If, however, the larva receives a second stimulus in the form of a second encounter with the older larva or extra conditioning, or both, its activity may be offset and it settles in the preferred highly conditioned lump.

This hypothesis is partly supported by my data, but much of it is only circumstantially supported and several tests are needed, all of which relate to an individual's behavior and activity. The fact that activity rhythms do differ among different ages of larvae is tentatively supported by the data in Chapter IV. However, high conditioning is a preferred stimulus and even active larvae will settle in it, although they may continue to sample their surroundings. The experiments in this paper support the idea that older larvae alter the behavior of younger larvae and lower the young larva's response to conditioning. However, the hypothesis that a second stimulus, in the form of extra conditioning or a second interaction with the older larva, is needed to increase the younger larva's response to conditioning is not well supported. An increased sample size may be needed to pull these relationships apart.

One of the major problems is that we cannot be sure that a second encounter with the older larva actually occurs, and if it does, whether it has any effect. This is a function of the experimental design. The first encounter occurs in the center of the dish

when both larvae are introduced into the apparatus. If the second encounter occurs, however, it would have to occur in the food lump in which the younger larva is residing, and cause it to leave that food lump and move to the highly conditioned one. Yet in Experiment II the data indicates that a larva's preference for conditioned food in which an older larva is residing is unaffected by that older larva. The situation in which an older larva moves to conditioned food containing a young resident was not tested and we do not know what the effect on either larva would be. This needs to be done.

We also do not know if any interaction occurs between two larvae in a conditioned food lump. Such a situation may buffer any interactions demonstrated to occur in the open (in the middle of the dish) and it is in fact, possible for two larvae not to encounter each other, at least directly, in a food lump. Tests are needed in which the experimental situation has been so altered as to maximize the chances of two larvae meeting in the food lumps. One possibility is to decrease the size of the food lumps. A better approach might be to so construct the conditioned food lump that only enough food is available for the survival of one larva and ask the question which larva is ejected.

The other part of the hypothesis that is not well supported is that extra conditioning may be a required stimulus to offset the lowered activity of the younger larva after an initial encounter with the older larva. This may be easily tested by the following comparison. In the final experiment of this paper group (1-0-0) received an initial encounter with the older larva but no extra conditioning or

second encounter. It was found that the 7 day old response curve does not significantly rise. That is, even when the older larva is removed the younger larva does not move to the conditioned food. This group should be rerun, but after about 4 days an even more highly conditioned lump would be added to the situation. If extra conditioning is a factor in offsetting the increased activity, the younger larvae should move to this new lump and remain there.

Finally we need more information about the general activity of these larvae. For example, the initial encounter described is between a 7 and a 13 day old larva, but the second encounter, if it is occurring, is between a 16 or 17 day old larva (the original 13 day old larva) and an 11 or 12 day old larva (the original 7 day old larva). The two situations may be totally different as to the effects on each larva. The effect of the initial encounter and the second encounter may be a function of the size difference between test larvae. This may be tested by systematically decreasing the age differences between test larvae.

The variability encountered in these experiments also needs to be investigated. Variability may be environmental, genetic, or both. Two things need to be done. First, is to begin manipulating the larva's environment to see what happens to the variability. For example, there were no controls in my experiments for the fact that some larvae were reared in more dense colony groups than were others or that older larvae in the colony are subjected to higher degrees of conditioning than were younger larvae. These two factors may have an effect on the activity levels and preference for varying degrees

of conditioning. If all larvae were reared as isolates before testing, this may result in different levels of variability.

A second approach is to artificially select for a "standard" population of Galleria so that the genetics of my experimental animal is under control. As an extension of this I would also like to select for a population of larvae that do not spin silk and begin asking questions about whether the behavioral response to conditioning is genetically related to the physical act of spinning.

Once these variables are under control, the next step would be to make predictions about the behavior of larger larval populations, and experimentally test the predictions.

CHAPTER VI

SUMMARY

The studies in this dissertation deal with the preference behavior of individual Galleria mellonella larvae for homotypically conditioned food, its relationship to spatial distribution, and the effects of degree of conditioning, developmental age, and presence of conspecifics on these preferences. A mechanism is postulated for the interaction of these variables with larval activity in determining spatial patterns. Also presented is an analytical technique for the analysis of multiple samples taken from the same individuals over time.

Isolate Behavior

1. Response of isolate larvae to homotypic conditioning is either random or attraction, depending on the degree of conditioning used.
2. Within a particular age of test larvae, response to conditioning increases as the degree of conditioning increases up to a certain level; the degree of conditioning being measured in terms of the number of days conditioning occurs, the age of the conditioning larva, or both. A threshold effect is

indicated, in which degrees of conditioning above a certain level do not elicit a greater response.

3. There appears to be an age difference in response to conditioning. Older larvae exhibit a greater response than younger larvae, at least initially, to the same degree of conditioning. However, these age differences cannot be separated from early experience differences of the larvae with colony density and colony conditioning, or from length of experience with the experimental situation.
4. Age differences in initial response to low versus high degrees of conditioning are more pronounced between young and old larvae. Older larvae exhibit higher discrimination between degrees of conditioning than do younger larvae.
5. A difference in activity, as measured by larval movement between food lumps, between young and older larvae was indicated. Both exhibit increased movement to conditioned food as conditioning increases, but older larvae do most such movement in the earlier periods of the experiment and young larvae move more 3 or 4 days later.
6. Activity differences are supported by the variance vectors for each group. As response to conditioning increases, the variances decrease, whereas the variances remain large when larval response to low conditioning is low. This indicates that more movement between food lumps occurs when one of the lumps is non-conditioned and the other has a low degree of conditioning. As conditioning increases, larvae select the

conditioned food and tend not to continue moving between food lumps.

7. Larval movements and preference for conditioning are affected by food source. Larvae exhibit an initial preference for deficient-conditioned food but move to normal non-conditioned food within 24 hours. The initial response to deficient-conditioned food is also initially lower than to normal-conditioned food. This may be a function of the conditioning itself or the food source.
8. Female (adult) Galleria avoid highly, larval-conditioned food for egg laying purposes and this was interpreted in terms of an ability to interpret high conditioning as high larval population and a behavior of laying eggs in situations where larval density is low.

Interactions Between Conspecifics and Biological Conditioning

9. Pairs of larvae initially distribute themselves randomly when placed in a situation where no previous conditioning has occurred. With time, however, significant aggregation occurs between 7+7, 7+11, or 11+11 day old larvae. Whenever a 13 day old larva is present, significant pairing does not occur.
10. Aggregation behavior is not due to some discrepancy in food lumps but is due to either an interaction between larvae, biological conditioning, or both. For example, pairing in the 7+11 day old situation is due to the 7 day old larva moving to the more highly conditioned food lump where the 11 day old

larva is residing. Similar movements do not occur to where a 13 day old larva is residing, probably a function of larval-larval intolerance, not conditioning.

11. When one of the food lumps is highly conditioned, aggregation behavior occurs in that lump, regardless of the age of the two test larvae. However, the response of individual larvae to the conditioning is affected by the presence of a conspecific.
12. If a conspecific is a resident in the conditioned food, the behavior of test larvae is the same as if the conspecific were not present. For example, a 7 day old larva's response to a conditioned food lump with a resident 13 day old larva in it is the same as a 7 day old's response to a conditioned food lump lacking a resident. This behavior is the same whether the food was low or high in conditioning.
13. When both test larvae are introduced into the experimental situation at the same time, their responses to conditioning are lowered if conditioning is high and unaffected if the conditioning is low, as measured against the response of isolates to similar degrees of conditioning. When response is affected, the response of the younger larva is more drastically lowered than is that of the older larva, but eventually increases with time.
14. The younger larva's response was manipulated by varying the age of the older conspecific and it was postulated that the behavior of the younger larva is a result of three variables: (1) the initial encounter with the older larva, (2) the extra

conditioning being done by the older larva, and (3) a second encounter with the older larva.

15. Experiments utilizing various combinations of these variables demonstrated that the initial encounter was the factor involved in the lowered initial preference for conditioning, but the effects of extra conditioning and a second interaction were inconclusive. An hypothesis was advanced relating the interaction between biological conditioning, larval-larval interactions, and larval activity rhythms to the observed spatial patterns of Galleria larvae and various tests proposed. A larger sample size may be needed to elucidate these variables Such a sample size may be calculated by $N = 3/\hat{w}(1-\hat{w})$, as discussed in Chapter III.

16. The general conclusion from all of these studies is that individual behavior and distribution is a function of degree of conditioning, age of the individual, presence and age of a conspecific, location of the conspecific, and possibly the activity of Galleria larvae.

17. Hotelling's One- and Two-Sample T^2 Statistic for Multivariate Analyses was utilized for the analysis of the data, with modifications for handling longitudinal data on individuals. This proved to be a very effective way of handling data on individual animals and for comparing the total response of organisms over time.

BIBLIOGRAPHY

BIBLIOGRAPHY

Adolph, E. F. 1931. The size of the body and the size of the environment in the growth of tadpoles. Biol. Bull. 61:350-375.

Alexander, Nancy J. 1970. A regulatory mechanism of ecdysone release in Galleria mellonella. J. Insect Physiol. 16: 277-289.

Alexander, P. and D. H. R. Barton. 1943. The excretion of ethylquinone by the flour beetle. Biochem. Jour. 37(4):463-465.

Allee, W. C. 1926. Studies in animal aggregations: causes and effects of bunching in land isopods. J. Exp. Zool. 45: 255-277.

Allee, W. C. 1927. Animal aggregations. Quart. Review Biol. 2: 367-398.

Allee, W. C. and J. F. Schuett. 1927. Studies in animal aggregations: the relation between mass of animals and resistance to colloidal silver. Biol. Bull. 53:301-317.

Allee, W. C. 1931. Animal Aggregations: A Study in General Sociology. Chicago: University of Chicago Press.

Allee, W. C. and J. R. Fowler. 1932. Studies in animal aggregations: further studies on oxygen consumption and autotomy of the brittle star, Ophioderma. J. Exp. Zool. 64:1-19.

Allee, W. C. 1934. Recent studies in mass physiology. Biol. Rev. 9:1-48.

Allee, W. C., E. S. Bowen, J. C. Welty, and R. Oesting. 1934. The effect of homotypic conditioning of water on the growth of fishes, and chemical studies of the factors involved. J. Exp. Zool. 68:183-213.

Allee, W. C., A. J. Finkel, and W. H. Hoskins. 1940. The growth of goldfish in homotypically conditioned water: a population study in mass physiology. J. Exp. Zool. 84:417-443.

Allee, W. C., Alfred E. Emerson, Orlando Park, and Thomas Park. 1949. Principles of Animal Ecology. Philadelphia and London: W. B. Saunders Company.

Andrews, J. E. 1921. Some experiments with the larva of the bee moth, Galleria mellonella L. Trans. Wis. Acad. Sci., Arts and Letters 20:255-261.

Asher, James H. 1970. Parthenogenesis and genetic variability. Ph.D. Dissertation. University of Michigan. Ann Arbor, Michigan.

Balazas, A. 1958. Nutritional and nervous factors in the adaptation of Galleria mellonella to artificial diet. Acta. Biol. 9:47-69.

Beck, Stanley D. 1960. Growth and development of the greater wax moth, Galleria mellonella (L.). (Lepidoptera: Galleriidae). Trans. Wis. Acad. Sci., Arts and Letters 49:137-148.

Bell, J. 1940. The heat production and oxygen consumption of Galleria mellonella at different constant temperatures. Physiol. Zool. 13:73-81.

Borchert, A. 1933. Zur Biologie de grossen Wachsmotte (Galleria mellonella). I. Uber morphologie und Entwicklungsdauer der larvae der grossen Wachsmotte. Zool. Jahr. Abteil. Anat. u. Ont. 57:105-115.

Bossert, William H. and Edward O. Wilson. 1963. The analysis of olfactory communication among animals. J. Theoret. Biol. 5:443-469.

Bovbjerg, Richard. 1952. Ecological aspects of dispersal of the snail Campeloma decisum. Ecology 33:169-176.

Bowen, E. S. 1921. The role of the sense organs in aggregations of Ameiurus melas. Ecol. Mono. 1:1-35.

Box, G. E. P. 1950. Problems in the analysis of growth and wear curves. Biometrics 6:362-389.

Brand, R. A. 1955. Abundance and activity of the woodmouse (Peromyscus leucopus noveboracensis) in relation to the characteristics of its habitat. Ph.D. Thesis. University of Michigan.

Braun. 1867. The bee moth. Amer. Bee Jour. 66:380-400.

Breukelman, John. 1932. Speed of toxic action of mercuric chloride on daphnids. Physiol. Zool. 5:207-218.

Brindley, H. H. 1910. Further notes on the procession of Cnethocampa pinivora. Proc. Camb. Phil. Soc. 15:576-587.

Brown, L. A. and A. M. Banta. 1935. Control of sex in Cladocera. Physiol. Zool. 8:138-155.

Brown, M. D. 1946. The growth of brown trout (Salmo trutta Linn.). I. Factors influencing the growth of trout fry. J. Exp. Biol. 22:118-144.

Brown, Margaret E. 1946. The growth of brown trout (Salmo trutta Linn.). I. I. Factors influencing the growth of trout fry. J. Exp. Biol. 22:118-129.

Brown, Margaret E. 1951. The growth of brown trout (Salmo trutta Linn.). IV. The effect of food and temperature on the survival and growth of fry. J. Exp. Biol. 28:473-491.

Brown, R. Z. 1953. Social behavior, reproduction, and population changes in the house mouse (Mus musculus L.). Ecol. Monogr. 23:217-240.

Burkett, B. N. 1962. Temperature block of spinning and development in Galleria mellonella (Lepidoptera). Amer. Zool. 2:396.

Butler, C. G., D. J. C. Fletcher, and Doreen Watler. 1969. Nest-entrance marking with phermones by the honeybee, Apis mellifera L., and by a wasp, Vespula vulgaris L. Anim. Behav. 17:142-147.

Calhoun, J. B. 1948. Mortality and movement of brown rats (Rattus norvegicus) in artificially super-saturated populations. Jour. Wildl. Mgt. 12:167-172.

Calhoun, J. B. 1950. The study of wild animals under controlled conditions. Annals New York Acad. Sci. 51:1113-1122.

Calhoun, J. B. 1952. The social aspects of population dynamics. Jour. Mamm. 33:138-159.

Calhoun, J. B. and W. L. Webb. 1953. Induced emigrations among small mammals. Science 117:358-360.

Carthy, J. D. 1951. The return of ants to their nest. Trans. 9th Int. Congr. Entom. 1:365-369.

Chapman, R. N. 1928. The quantitative analysis of environmental factors. Ecology 9:111-122.

Chase, Ruth W. 1921. The length of life of the larva of the wax moth, Galleria mellonella L., in its different stadia. Trans. Wis. Acad. Sci., Arts and Letters 20:263-267.

Christian, J. J. 1950. The adren-pituitary system and population cycles in mammals. J. Mamm. 31:247-259.

Christian, J. J. 1970. Social subordination, population density, and mammalian evolution. Science 168:84-90.

Chiang, H. C. and A. C. Hodson. 1950. An analytical study of population growth in Drosophila melanogaster. Ecol. Monogr. 20:173-206.

Chitty, D. 1955. Adverse effects of population density upon the viability of later generations. In the numbers of man and animals. Edited by J. B. Cragg and N. W. Pirce. Edinburgh: Oliver and Boyd, Ltd., 57-67.

Chitty, D. 1960. Population processes in the vole and their relevance to general theory. Can. J. Zool. 38:99-113.

Cochran, W. G. 1947. Some consequences when the assumptions for the analysis of variance are not satisfied. Biometrics 3:22-38.

Cole, H. A. and E. W. Knight-Jones. 1949. The settling behavior of larvae of the European flat oyster, Ostrea eduli L., and its influence on methods of cultivation and spat collection. Fishery Invest. London, ser 2. 17(3):59 pp.

Cole, L. C. 1946. A study of the dryptozoa of an Illinois' woodland. Ecol. Monogr. 16:49-86.

Collias, Nicholas E. 1964. The evolution of nests and nest building in birds. Amer. Zool. 4:175-190.

Crombe, A. C. 1942. The effect of crowding upon the oviposition of grain-infesting insects. J. Exper. Biol. 19:311-340.

Dadd, R. H. 1964. A study of carbohydrate and lipid nutrition in the wax moth, Galleria mellonella (L.), using partially synthetic diets. J. Insect Physiol. 10:161-178.

Dadd, R. H. 1966. Beeswax in the nutrition of the wax moth, Galleria mellonella (L.). J. Insect Physiol. 12:1479-1492.

Davis, D. E. 1958. The role of density in aggressive behavior in house mice. Anim. Behav. 6:207-211.

Davis, D. E. and J. J. Christian. 1956. Changes in Norway rat populations induced by introduction of rats. J. Wildl. Mgt. 20: 378-383.

Dawson, P. S. 1964. An interesting behavioral phenomenon in Tribolium confusum. Tribolium Inform. Bull. 7:50-52.

Dickman, A. 1933. Studies on the wax moth Galleria mellonella with particular reference to the digestion of wax by the larvae. J. Cell and Comp. Physiol. 3:223-246.

Doull, K M. 1953. Phase coloration in lepidopterous larvae. London. Nature 172:813-814.

Dryl, S. 1963. Contributions to mechanism of chemotactic response in Paramecium caudatum. Anim. Behav. 11:393-396.

Drzewina, A. and G. Bohn. 1921. Variations de la susceptibilite aux agents mocifs avec le nombre des animaux traites. C. R. Acad. Sci. 172:485-487.

Drzewina, A. and G. Bohn. 1926. Activation par la lumiere des effets de l'argent sur convoluta. C. R. Acad. Sci. 183:677-679.

Durbin, J. and G. S. Watson. 1950. Testing for serial correlation in least squares regression. I. Biometrika 37:409-428.

Durbin, J. and G. S. Watson. 1951. Testing for serial correlation in least squares regression. II. Biometrika 38:159-178.

Dutky, S. R., J. V. Thompson, and George E. Cantwell. 1962. A technique for mass rearing the greater wax moth. Proc. Ent. Soc. Wash. 64:56-58.

Edmondson, W. T. 1945. Ecological studies of sessile rotatoria. II. Dynamics of populations and social structures. Ecol. Monogr. 15:143-172.

Edney, E. B. 1954. Woodlice and the land habitat. Biol. Rev. 29: 185-219.

Edwards, J. S. 1966. Neural control of metamorphosis in Galleria mellonella (Lepidoptera). J. Insect Physiol. 12:1423-1433.

Edwards, T. G. 1910. On the procession and pupation of the larva of Cnethocampa pinivora. Proc. Camb. Phil. Soc. 15:431-436.

Ellis, P. E. 1953. Social aggregation and gregarious behaviors in hoppers of Locusta migratoria migratorivides. (R. and F.). Behavior 5:225-260.

El-Sawaf, S. K. 1950. The life history of the greater wax moth (Galleria mellonella L.) in Egypt, with special reference to the morphology of the mature larva. Bull. Soc. Fouad. Ent. 34:247-297.

Faure, J. C. 1932. The phases of locusts in South Africa. Bull. Ent. Res. 23:293-428.

Fitch, John H. 1975. Behavioral habitat selection as a factor in the distribution of woodland deermice, Peromyscus maniculatus gracilis, in the Kingston Plains of Northern Michigan. Ph.D. Dissertation. Michigan State University.

Fraenkel, G. S. and D. L. Gunn. 1961. The Orientation of Animals. New York: Dover Publications.

Free, J. B. 1961. The stimuli releasing the stinging response of honeybees. Anim. Behav. 9(3-4):193-196.

Friedlander, C. P. 1964. Thigmokinesis in woodlice. Anim. Behav. 12:164-174.

Gee, J. M. 1965. Chemicals stimulation of settlement in larvae of Spirorbis rupestris (Serpulidae). Anim. Behav. 13(1):181-186.

Ghent, Arthur W. 1963. Studies of behavior of the Tribolium flour beetles. I. Contrasting responses of T. castaneum and T. confusum to fresh and conditioned flour. Ecology 44(2): 269-283.

Ghent, Arthur W. 1966. Studies of behavior of the Tribolium flour beetles. II. Distributions in depth of T. castaneum and T. confusum in fractionable shell vials. Ecology 47(3):355-367.

Gill, J. L. and H. D. Hafs. 1971. Analysis of repeated measurements of animals. J. Anim. Sci. 33:331-336.

Graham, W. M. 1958. Temperature preference determination using Tribolium. Anim. Behav. 6:231-237.

Gunn, D. L. and D. P. Prelou. 1940. The humidity behavior of the mealworm beetle, Tenebrio molitor. III. The mechanism of the reaction. J. Exp. Biol. 17:307-316.

Harris, V. T. 1956. An experimental study of habitat selection by prairie and forest races of the deer mouse. Peromyscus maniculatus. Contrib. Lab. Vert. Biol. Univ. of Michigan 56:53 pp.

Harshbarger, John C. and B. Shain Moore. 1966. Some effects of x-irradiation on larvae of Galleria mellonella. J. Invert. Path. 8(2):277-279.

Haydak, Mykola H. 1936. Is wax a necessary constituent of the diet of wax moth larvae? Ann. Ent. Soc. Amer. 29:581-588.

Haydak, Mykola H. 1940. The length of development of the greater wax moth. Science 91:525.

Heatwole, Harold. 1962. Environmental factors influencing local distribution and activity of the salamander, Plethodon cinereus. Ecology 43:460-472.

Hotelling, Harold. 1931. The generalization of student's ratio. Ann. of Math. Stat. 2:360-378.

Howard, Eliot. 1920. Territory in Bird Life. New York: Atheneum.

Imms, A. D. 1948. A General Textbook of Entomology. New York: Dutton.

Issakowitsch, A. 1905. Geschlechtsbestimmende ursachen bei den daphniden. Biol. Centralbl. 25:529-536.

Ito, Koichi and William Schull. 1964. On the robustness of the T_0^2 test in multivariate analysis of variance when variance-covariance matrices are not equal. Biometrika 51:71-82.

Jacobson, Martin, Meyer Schwarz, and Rolland M. Water. 1970. Gypsy moth sex attractants: A reinvestigation. J. Econ. Ent. 63(3): 943-945.

Jespersen, P. 1924. The frequency of birds over the high Atlantic Ocean. London. Nature 114:281-283.

Johnson, R. P. 1973. Scent marking in mammals. Anim. Behav. 21: 521-535.

Johnston, James W., David G. Moulton, and Amos Tuck. 1970. Communication by Chemical Signals, Vol. I. New York: Appleton-Century-Crofts.

Karlson, P. and A. Butenandt. 1959. Phermones (ectohormones) in insects. Ann. Rev. Entom. 4:39-58.

Kaston, B. J. 1964. The evolution of spider webs. Amer. Zool. 4:191-207.

Knight-Jones, E. W. and J. P. Stevenson. 1951. Gregariousness during settlement in the barnacle, Elminuis modestus Darwin. J. Mar. Biol. Ass. U. K. 29:281-297.

Krebs, C. J. 1966. Demographic changes in fluctuating populations of Microtus californicus. Ecol. Monogr. 36:239-273.

Krebs, Charles J., Barry L. Keller, and Robert H. Tamarin. 1969. Microtus population biology: Demographic changes in fluctuating populations of M. ochrogaster and M. pennsylvanicus in Southern Indiana. Ecology 50(40):587-607.

Lack, David. 1954. The Natural Regulation of Animal Numbers. Oxford: Clarendon Press.

Legay, J. M. and J. L. Chasse. 1964. Etude experimental de la sortie d'une enciente chez Tribolium confusum. Acad. Sci. (Paris), Compt. rend. 258:717-719.

Lerner, I. M. and N. Inouze. 1968. Behavior genetics with special reference to maze-running of Tribolium. In Dronamraju K. R. (ed.). Haldane and Modern Biology. Johns Hopkins Press.

Loconti, Joseph D. and Louis M. Roth. 1953. Composition of the odorous secretion of Tribolium castaneum. Ent. Soc. of Amer., Annals 46:281-289.

Loomis, W. F. and H. M. Lenhoff. 1956. Growth and sexual differentiation of hydra in mass culture. J. Exp. Zool. 132:555-568.

Long, D. B. 1953. Effects of population density on larvae of Lepidoptera. Trans. R. Ent. Soc. Lond. 104:544-585.

Long, D. B. 1955. Observations on sub-social behavior in two species of Lepidoptera larvae, Pieris brassicae L. and Plusia gamma L. Trans. R. Ent. Soc. Lond. 106:421-437.

Loschiavo, S. R. 1952. A study of some food preferences of Tribolium confusum Duv. Cereal Chemistry 29:91-107.

Lwengood, W. F. 1937. An experimental analysis of certain factors affecting growth of goldfishes in homotypically conditioned water. Copeia. 81-88.

Magnuson, John J. 1962. An analysis of aggressive behavior, growth, and competition for food and space in Medaka (Oryzias latipes) (Pisce: Cryprinodontidae). Canadian J. of Zool. 40:313-363.

Maschwitz, Ulrich W. 1964. Alarm substances and alarm behavior in social hymenoptera. Nature 204:324-327.

McDonald, D. J. and L. Fitting. 1965. Activity differences in black and wild type strains of Tribolium confusum. Tribolium Inform. Bull. 8:124-125.

McDonald, Daniel J. 1968. Mobility in Tribolium confusum. Ecology 49:770-771.

Milum, V. G. 1952a. Characters and habits of moth larvae infesting honeybee combs. Amer. Bee Jour. 92:200-220.

Milum, V. G. 1952b. Descriptions and habits of the adults of some moths whose larvae infest combs of honeybees. Amer. Bee Jour. 92:155-156.

Milum, B. G. and H. W. Geuther. 1935. Observations on the biology of the greater wax moth, Galleria mellonella L. J. Econ. Ent. 28:576-578.

Morrison, Donald F. 1967. Multivariate Statistical Methods. New York: McGraw-Hill Book Company.

Myers, Judith and Charles J. Krebs. 1974. Population cycles in rodents. Sci. Amer. 230:38-46.

Naylor, Alfred F. 1959. An experimental analysis of dispersal in the flour beetle, Tribolium confusum. Ecology 40:453-465.

Naylor, Alfred F. 1961. Dispersal in the red flour beetle Tribolium castaneum (Tenebrionidae). Ecology 42(2):231-237.

Naylor, Alfred F. 1965. Dispersal responses of female flour beetles, Tribolium confusum, to presence of larvae. Ecology 46: 341-343.

Niemierko, W. and S. Cepelewicz. 1950. Studies in the biochemistry of the wax moth (Galleria mellonella). 1. Growth of the larvae and their chemical composition. Acta Biol. Exp. 15(4):57-68.

Niemierko, W. and P. Wlodawer. 1950. ...2. Utilization of wax constituents by the larvae. Acta Biol. Exp. 15(5):69-78.

Niemierko, W. and P. Wlodawer. 1952. ...7. The digestion of wax and utilization of unsaponifiable substances by larvae. Acta Biol. Exp. 16(12):157-170.

Niemierko, W. and L. Wojtczak. 1950. 3. Oxygen consumption of the larvae during starvation. Acta Biol. Exp. 15(6):79-90.

Oertel, E. 1962. Behavior studies of the greater wax moth. XI Intern. Kongress fur. Entomologie, Wien. 532-536.

Oertel, E. 1963. Greater wax moth develops on bumble bee cells. J. Econ. Ent. 56(4):543-544.

Ogden, John C. 1969. Effect of components of conditioned medium on behavior in Tribolium confusum. Physiol. Zool. 42:266-274.

Ogden, John C. 1970. Aspects of dispersal in Tribolium flour beetles. Physiol. Zool. 43:124-131.

Ogden, John C. 1970b. Artificial selection for dispersal in flour beetles (Tenebrionidae: Tribolium). Ecology 51:130-133.

Orgain, H. and M. W. Schein. 1953. A preliminary analysis of the physical environment of the Norway rat. Ecology 34:467-473.

Paddock, F. B. 1913. The life history and control of the bee-moth or wax-worm. Texas Agri. Exper. Station Bull. 158:15-30.

Paddock, F. B. 1930. The bee moths. J. Econ. Ent. 23:422-428.

Park, Thomas. 1932. Studies in population physiology. The relation of numbers to initial population growth in the flour beetle Tribolium confusum. Ecology 13:172-181.

Park, Thomas. 1933. ...II. Factors regulating initial growth of Tribolium confusum populations. J. Exp. Zool. 65:17-42.

Park, Thomas. 1934. ...III. The effect of conditioned flour upon the productivity and population decline of Tribolium confusum. J. Exp. Zool. 68(2):167-182.

Park, Thomas. 1935. ...IV. Some physiological effects of conditioned flour upon Tribolium confusum Duval and its populations. Physiol. Zool. 8:91-115.

Park, Thomas. 1936a. ...V. The oxygen consumption of the flour beetle, Tribolium confusum Duval. J. of Cellular and Comp. Physiol. 7(3):313-323.

Park, Thomas. 1936b. ...VI. The effect of differentially conditioned flour upon the fecundity and fertility of Tribolium confusum Duval. J. Exp. Zool. 73(3):393-404.

Park, Thomas and Woollcott. 1937. ...VII. The relation of environmental conditioning to the decline of Tribolium confusum populations. Physiol. Zool. 10:197-211.

Park, Thomas. 1938. Studies in population physiology. VIII. The effects of larval population density on the post-embryonic development of the flour beetle T. confusum. J. Exp. Zool. 79:51-70.

Park, Thomas, E. V. Miller, and C. Z. Lutherman. 1939. ...IX. The effect of imago population density on the duration of the larval and pupal stages of Tribolium confusum Duval. Ecology 20:365-373.

Park, Thomas and W. Burrows. 1942. The reproduction of Tribolium confusum Duval in a semisynthetic wooddust medium. Physiol. Zool. 15:476-484.

Park, Thomas. 1948. Experimental studies of interspecies competition. 1. Competition between populations of the flour beetles, Tribolium confusum Duval and Tribolium castaneum Herbst. Ecol. Monogr. 18:265-307.

Parker, J. S. 1930. Some effects of temperature and moisture upon Melanoplus mexicanus saussure and Camnula pellucida Scudder (Orthoptera). Univ. Montana Agri. Expt. Sta. Bull. No. 223.

Pearl, Raymond. 1927. The growth of populations. Quart. Rev. Biol. 2:530-548.

Peebles, Florence. 1929. Growth regulating substances in echinoderm larvae. Biol. Bull. 57:176-187.

Pfeiffer, W. 1963. Alarm substances. Experientia. 19:113-168.

Pipa, R. L. 1963. Studies on the hexapod nervous system. VI. Ventral nerve cord shortening; a metamorphic process in Galleria mellonella (L.) (Lepidoptera, Pyrallidae). Biol. Bull. Woods Hole. 124:293-302.

Plath, O. E. 1934. Bumblebees and Their Ways. New York: Macmillan.

Prus, T. 1963. Search for methods to investigate mobility in Tribolium. Ecology 44:801-803.

Przelecka, A. 1956. Studies on the biochemistry of wax moth (Galleria mellonella L.). 14. Cytochemical study of phospholipids in the intestinal tract of wax moth larvae. Acta Biol. Exp. 17(1):231-234.

Rathcke, Beverly J. and Robert W. Poole. 1975. Coevolutionary race continues: butterfly larval adaptation to plant trichomes. Science 187:175-176.

Regnies, Fred E. and John H. Law. 1968. Insect phermones. J. of Lipid Res. 9:541-551.

Reynierse, James H. 1967. Aggregation formation in planaria, Phagocata gracilis and Cura foremani: species differentiation. Anim. Behav. 15:270-272.

Reynierse, James H., Kathryn K. Gleason, and Robert Ottermann. 1969. Mechanisms producing aggregations in planaria. Anim. Behav. 17(1):47-63.

Rich, Earl R. 1956. Egg cannibalism and fecundity in Tribolium. Ecology 37:109-120.

Richards, C. M. 1958. The inhibition of growth in crowded Rana pipiens tadpoles. Physiol. Zool. 31:138-151.

Robertson, T. B. 1921. Experimental studies on cellular manipulation. II. The influence of mutual antiquity upon reproductive rate and the part played therein by the "x-substance" in bacterized infusions which stimulates the multiplication of infusoria. Biochem. Jour. 15:595-611, 612-619.

Rohlf, James F. and Robert R. Sokal. 1969. Statistical Tables. San Francisco: W. H. Freeman and Company.

Rose, S. Meryl. 1959. Failure of survival of slowly growing members of a population. Science 129:1026.

Rose, S. M. 1960. A feedback mechanism of growth control in tadpoles. Ecology 41:188-199.

Roth, Louis M. and Ruth B. Howland. 1941. Studies on the gaseous secretion of Tribolium confusum Duval. I. Abnormalities produced in Tribolium confusum Duval by exposure to a secretion given off by the adults. Ann. Ent. Soc. Am. 34(1):151-175.

Roth, Louis M. 1943. Studies on the gaseous secretion of Tribolium confusum Duval. II. The odoriferous glands of Tribolium confusum. Ent. Soc. of Amer., Annals 36:397-424.

Salt, George and F. S. J. Hollick. 1946. Studies of wireworm populations. II. Spatial distribution. J. Exp. Biol. 23:1-46.

Sharp, David T. and I. E. Gray. 1962. Studies on factors affecting the local distribution of two sea urchins, Arbacia punctulata and Lytechinus variegatus. Ecology 43:309-313.

Shaw, Gretchen. 1932. The effect of biologically conditioned water upon rate of growth in fishes and amphibia. Ecology 13: 263-278.

Shelford, V. E. 1913. The reactions of certain animals to gradients of evaporating power of air. A study in experimental ecology. Biol. Bull. 25:70-120.

Silverstein, Robert M., Otto J. Rodin, Wendell E. Burkholder, and John E. Gorman. 1967. Sex attractant of the black carpet beetle. Science 157:85-86.

Silverstein, Robert M., Robert G. Brownlee, Thomas E. Bellas, David L. Wood, and Lloyd E. Browne. 1967. Brevicomin: Principal sex attractant in the frass of the female pine beetle. Science 159:889-890.

Skaife, S. H. 1954. African Insect Life. London: Longmans Green.

Sladen, F. W. L. 1912. The Bumble-Bee. London: Macmillan.

Smith, G. 1915. The life-cycle of Cladocera, with remarks on the physiology of growth and reproduction in crustacea. Proc. Roy. Soc. London B. 88:418-535.

Smith, Stephen D. 1969. The effects of crowding on larvae of the meal moth, Ephestia kuhniella. J. Exp. Zool. 170:193-203.

Skal, Robert R. and James F. Rohlf. 1969. Biometry. San Francisco: W. H. Freeman and Company.

Sokoloff, A. 1966. A behavioral difference of two mutant strains in Tribolium castaneum. Tribolium Inform. Bull. 9:109-110.

Stanley, J. 1932. A mathematical theory of the growth of populations of the flour beetle Tribolium confusum Duval. Can. J. Research 6:632-671.

Stanley, John. 1949. A mathematical theory of the growth of populations of the flour beetle, Tribolium confusum Duv. VII. A study of the "Re-tunneling Problem." Ecology 30(2): 209-222.

Stickle, L. F. 1946. The source of animals moving into a depopulated area. J. Mamm. 27:301-307.

Strecker, R. L. 1954. Regulatory mechanics in house mouse populations--the effect of limited food supply on a confined population. Ecology 34:375-385.

Stuart, C. A. and A. M. Banta. 1931. Available bacteria and the sex ratio in Moina. Physiol. Zool. 4:72-86.

Surtees, Gordon. 1963a. Laboratory studies on dispersion behavior of adult beetles in grain. I. The grain weevil, Sitophilus granarius (L.) (Coleoptera, Curculionidae). Bull. Ent. Res. 54:147-159.

Surtees, Gordon. 1963b. ...II. The saw-toothed grain beetle, Oryzaephilus surinamensis (L.) (Coleoptera, Silvanidae). Bull. Ent. Res. 54:285-296.

Surtees, Gordon. 1963c. ...III. Tribolium castaneum (Hbst.) (Coleoptera, Tenebrionidae) and Cryptolestes ferrugineus (Steph.) (Coleoptera, Cucujidae). Bull. Ent. Res. 54:297-306.

Surtees, Gordon. 1964a. ...VI. Three-dimensional analysis of dispersion of five species in a uniform bulk. Bull. Ent. Res. 55:161-171.

Surtees, Gordon. 1964b. ...VII. The effect of isolated pockets of damp and mouldy grain on Tribolium castaneum (Hbst.) (Coleoptera, Tenebrionidae). Bull. Ent. Res. 55:327-337.

Surtees, Gordon. 1964c. ...VIII. Spontaneous activity in three species and a new approach to analysis of kinesis mechanisms. Anim. Behav. 12:374-377.

Surtees, Gordon. 1964d. ...IX. Some differences in behavior of mated and unmated grain weevils. Entom. Monthly Mag. 100: 105-108.

Surtees, Gordon. 1964e. ...XI. Some effects of temperature. Anim. Behav. 12:378-381.

Surtees, Gordon. 1964f. ...XII. The effect of isolated pockets of damp and mouldy wheat on Cryptolestes ferrugineus (Steph.) (Coleoptera, Cucujidae). Bull. Ent. Res. 55:673-680.

Talbot, Mayr. 1934. Distribution of ant species in the Chicago region with reference to ecological factors and physiological toleration. Ecology 15:416-439.

Taylor, Sneyd J. 1931. A case of "assembling" in the phasmidae. Proc. Ent. Soc. London. 6:45.

Terman, C. Richard. 1961. Some dynamics of spatial distribution within semi-natural populations of prairie deermice. Ecology 42:288-302.

Thorpe, W. H., A. C. Crombie, R. Hill, and J. H. Darrah. 1946. The behavior of wireworms in response to chemical stimulation. J. Exp. Biol. 23:234-267.

Uvarov, B. P. 1928. Grasshoppers and Locusts. London: Imp. Bur. Entom. 352 pp.

Van der Kloot, William G. and Carroll M. Williams. 1953. Cocoon construction by the Cecropia silkworm. I. The role of the external environment. Behaviour 2+3:141-156.

Van der Kloot, William G. and Carroll M. Williams. 1953b. ...II. The role of the internal environment. Behaviour 5:157-174.

von Frisch, Karl. 1974. Animal Architecture. New York and London: Harcourt Brace Jovanovich.

Walrecht, B. J., Jr. 1953. Similarity of the building instinct of social bees and wasps. Levende Natur. 61(3):70-72.

Walter, H. E. 1907. The reactions of planaria to light. J. Exp. Zool. 5:35-162.

Warburg, M. R. 1964. The response of isopods towards temperature, humidity, and light. Anim. Behav. 12:175-186.

Waterhouse. 1959. Axenic culture of wax moths for digestion studies. Ann. N. Y. Acad. Sci. 77:283-289.

Watson, H. S. 1953. Serial correlation in regression analysis. I. Biometrika. 42:327-341.

Way, M. J. and M. Cammell. 1970. Aggregation behavior in relation to food utilization by aphids. In Animal Populations in Relation to Their Food Resources (ed. Adam Watson). 1970. Bkackwell Scientific Publ. Oxford and Edinburgh.

Wecker, Stanley C. 1963. The role of early experience in habitat selection by the prairie deer mouse, Peromyscus maniculatus bairdi. Ecol. Monogr. 33:307-325.

Wellington, W. G. 1948. The light reactions of the spruce budworm, Choristoneura fumiferana Clemens (Lepidoptera: Tortruidae). Canad. Ent. 80:56-82.

Wellington, W. G. 1957. Individual differences as a factor in population dynamics: the development of a problem. Canad. J. Zool. 35:293-323.

Wellington, W. G. 1960. Qualitative changes in natural populations during changes in abundance. Canad. J. Zool. 38:289-314.

Welty, J. C. 1934. Experimental explorations into group behavior of fishes: a study of the influence of the group on individual behavior. Physiol. Zool. 7.

West, L. B. 1961. The nature of growth inhibitory material from crowded Rana pipiens tadpoles. Physiol. Zool. 33:232-239.

Whitcomb, Warren. 1936. The wax moth and its control. U.S. Dept. of Agri. Circular No. 386:1-10.

Whitcomb, Warren. 1942. The wax moth and its control. U.S. Dept. of Agri. Circular No. 386:1-11.

Williams, O. L. 1934. Chapter 4, Termites and Termite Control. University of California Press. Berkeley, California.

Witt, Peter N. and Charles F. Reed. 1965. Spider-web building. Measurement of web geometry identifies components in a complex invertebrate behavior pattern. Science 149:1190-1197.

Wojtczak, L. 1952a. Studies on the biochemistry of the wax moth (Galleria mellonella). 10. Respiratory enzymes of the larva. Acta Biol. Exp. 16(15):199-222.

Wojtczak, L. 1952b. ...11. Respiratory enzymes in development. Acta Biol. Exp. 16(16):223-238.

Wojtczak, L. 1952c. ...12. Respiratory enzymes of the larva during starvation. Acta Biol. Exp. 16(17):239-246.

Woolever, Patricia and Rudolph Pipa. 1970. Spatial and feeding requirements for pupation of last instar larval Galleria mellonella Lepidoptera. J. Insect Physiol. 16:251-262.

Wynne-Edwards, V. C. 1962. Animal Dispersion in Relation to Social Behavior. Edinburgh and London: Oliver and Boyd.

Young, Roger G. 1961. The effects of dietary beeswax and wax components on the larvae of the greater wax moth, Galleria mellonella (L.). Ann. Entom. Soc. Amer. 54:657-659.

Young, Roger G. 1964. Digestion of wax by the greater wax moth, Galleria mellonella. Ann. Entom. Soc. Amer. 57:325-327.

Zielinska, A. M. 1952. Studies on the biochemistry of the wax moth (Galleria mellonella). 8. Nitrogen metabolism of the larvae. Acta Biol. Exper. 16(13):171-186.

Zromska-Rudyka, H. 1966. An appreciation of the laboratory set for investigating the emigratory activity of Tribolium. Ekologic Polska (A) 14:125-132.