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THE INHERITANCE OF DIAPAUSE CHARACTERISTICS IN THE FLESH FLY, SARCOPHAGA BULLATA

The Ohio State University

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THE INHERITANCE OF DIAPAUSE CHARACTERISTICS
IN THE FLESH FLY, SARCOPHAGA BULLATA

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

Vincent C. Henrich, B.S., M.Sc.

* * * * *

The Ohio State University
1982

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To my wife, Deborah, who
has made it worthwhile
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I owe much of the credit for my professional and personal development to people who have taken the time and spent some energy on my behalf. I thank the members of my committee, Dr. Denlinger, Dr. Rothenbuhler, Dr. House, and Dr. Skavaril, for the inspiration, encouragement, and expertise, that made this a successful endeavor. I also thank many friends over many years for their love and encouragement, and I thank my parents for the importance they have always placed on my well-being and their willingness to sacrifice for it.
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Chapter I
LITERATURE REVIEW

A. Introduction

Many insects enter a state of developmental arrest called diapause and thereby survive harsh climatic conditions associated with winter in temperate regions. For a given species, diapause always occurs at the same stage, but has been associated with all phases of the insect life cycle in various species. The phenology of the diapause trait varies widely among species, an indication that diapause has evolved independently on several occasions.

Whether an insect enters diapause or develops continuously depends upon the presence of environmental cues such as daylength that portend winter. Exposure to the appropriate environmental cues during a critical period in the life cycle programs the insect to enter diapause at a later developmental stage.

While the actual manifestation of diapause is environmentally dictated, the underlying genetic capability greatly affects the diapause response in a particular environment. Several lines of evidence indicate that genetic factors exert an influence upon diapause incidence and diapause duration. Supportive data have been obtained from (1) response of diapause traits to selection, (2) inheritance studies of diapause traits utilizing strains which show different
B. Studies with Natural Populations

1. Measurements of incidence and critical photoperiod

Almost all insects that live in temperate regions enter diapause in response to daylength. The appropriate photoperiod for diapause induction varies by geographical latitude. This variability has served as the source material for a number of studies of the genetic basis of diapause and its adaptive importance in insects (Tauber and Tauber, 1981). Diapause capability is conveniently measured by determining the point of transition between daylengths that evokes no diapause response and those that evoke a high response. This transition usually extends over a small range of daylengths and is called the critical photoperiod.

Bradshaw (1976) investigated the variation in critical photoperiod among several strains of the pitcher plant mosquito, *Wyeomyia smithii*, isolated from a variety of latitudinal, altitudinal, and longitudinal clines. Over 80% of the variation in critical photoperiod between these strains was accounted for by latitudinal differences.

Danilevskii (1965) analyzed the critical photoperiod in three natural strains of *Acronycta rumicis*. Lines derived from each strain consistently revealed that critical photoperiod was greater in northern strains than in southern strains and did not vary by more than an hour with any strain. Crosses between the northernmost and
southernmost strain produced hybrids that showed an intermediate critical photoperiod. Backcross and $F_2$ data were consistent with the hypothesis that critical photoperiod is inherited as an additive, quantitative trait with little or no dominance. Danilevskii also analyzed strains of *Pieris brassicae* and *Pyrausta nubilalis* and these yielded similar patterns. An additive model with no dominance easily accounts for the continuous variation in critical photoperiod seen across the geographical range of these species.

The milkweed bug, *Oncopeltus fasciatus*, also shows a continuous pattern of response to photoperiod as a function of latitudinal origin (Dingle, 1975; Dingle et al., 1977). In response to short days, adults enter a reproductive diapause, grow wings suitable for flight, and migrate long distances to more hospitable locations. Selection was rapid for low diapause, measured by the number of days before first egg deposition by the adult female.

The decrease in diapause incidence resulted from a change in the critical photoperiod. In a line selected for high diapause, the age until first reproduction increased through two generations, then decreased dramatically. Dingle suggests that early reproduction confers an advantage for survival, except of course, as winter nears. The interaction between diapause tendencies and the environment is maintained by a genetic "rheostat". In supportive environments, early reproduction is favored, but the capacity for delayed reproduction exists and is utilized in less favorable environments.
Continuous variation in critical photoperiod cannot be construed as the simple consequence of additive polygenic inheritance. Strains of *Drosophila littoralis* show a continuous pattern of variability in critical photoperiod across the latitudinal range of the species (Laakovara et al., 1972). Analysis of the progeny from several interstrain crosses reveals that hybrids tend to show "northern" characteristics. In other words, adult reproductive diapause occurs in response to relatively long photoperiods. Segregation patterns for critical photoperiod in the $F_2$ generation from each inter-strain cross correlated well with the differences at a single, autosomal locus (Lumme et al., 1975). Lumme attributes the observations to a supergene containing several functional subunits that allows for a continuous pattern of variation in critical photoperiod depending upon the allelic combination. The dominance of "northern" characters allows southern populations to interbreed with "northern" ones while insuring that hybrids make an early diapause response and survive through the winter. Lumme (1981) has succeeded in defining a locus in the *D. littoralis* genome that shows characteristics consistent with the supergene model.

Interstrain crosses have been utilized to analyze the inheritance patterns of several other species of Diptera. Ring (1971) crossed two strains of *Lucilia caesar* which show different responses to the same photoperiod. The $F_1$ and $F_2$ progeny obtained from these crosses showed a response that was roughly intermediate, and variation patterns are consistent with a polygenic mode of inheritance. Similar results have been obtained in *Sarcophaga peregrina* (Kurahashi and Ohtaki, 1977)
utilizing a temperate strain which enters pupal diapause and a tropical strain that shows no photoperiodic response.

Larval diapause in *Calliphora vicina* depends upon the female parent. The $F_1$ progeny from a mating between members of a high and low diapausing strain tended to resemble the characteristics of the female line (Vinogradova and Tsutskova, 1978). Whether the maternal inheritance is genetically based in the egg or rooted in physiologic events that occur in the adult female has not been discerned.

By correlating diapause tendencies among progeny with parental and grandparental tendencies in *Pionea forficalis*, King (1974) concluded that the characteristics of the offspring correlate most strongly with traits in the male line. The adaptive significance of the male line upon the seasonal phenology of diapause remains unexplained for this species.

The sexes often show different diapause tendencies (Ring, 1971; Helle, 1968; Denlinger, 1972b). Whether sex-based differences arise from sex-linked loci or from secondary sex differences has not been ascertained for any species. In the European corn borer, *Ostrinia nubilalis*, inheritance patterns for diapause incidence in several photoperiodic regimes yield data consistent with a sex-linked locus hypothesis (Reed et al., 1981).

The most revealing genetic studies with natural strains have been performed in the silkworm, *Bombyx mori* (reviewed by Lees, 1955). Differences in photoperiodic response among several strains correlated with three sex-linked alleles (at a common locus) and three autosomal
dominant loci. The differences were large enough to result in voltinistic differences between the strains. The complexity of the diapause response, particularly the importance of genetic-environmental interactions becomes apparent when analyzing the inheritance pattern of Bombyx. As a result of genetic differences, some larvae respond to a certain photoperiod as short day, while others perceive the same photoperiod as long day. When an eventual female encounters a subjective long day condition, she produces eggs that will enter diapause. Conversely, an eventual female exposed as a larva to short days will not produce diapausing eggs. In the case of mixed batches, the complexity is further realized. Some eggs will enter diapause and some will not, even when developing within the same maternal environment. This variability indicates possible genetic differences that have not been described in Bombyx.

Extremely large differences in critical photoperiod can result in differences in voltinistic characteristics among subpopulations. Ultimately, these populations could become temporally isolated to the point that they evolve as separate species. A prerequisite for such sympatric speciation is that the genetic basis for the isolating mechanisms be relatively simple (Maynard Smith, 1966; Bush, 1975). Two sibling species that occupy the same geographical location have been studied in this context (Tauber and Tauber, 1973). Chrysopa downesi enters adult reproductive diapause in response to relatively long daylengths and is therefore, univoltine. On the other hand, C. carnea responds to relatively short daylengths and is multivoltine. Analysis
of $F_1$, $F_2$, and backcross progeny from inter-special crosses revealed that the differences in critical photoperiod correlates with two autosomal loci, both having recessive alleles for the long critical photoperiodic response (Tauber et al., 1977; Tauber and Tauber, 1979). Differences in response as a consequence of relatively simple genetic differences have apparently led to reproductive isolation and speciation.

2. Diapause duration in natural strains

The incidence of diapause and critical photoperiod are not the only quantifiable traits associated with developmental arrest. Differences in diapause duration have also been correlated with latitudinal origin.

The same strains of *Wyeomyia smithii* which vary in critical photoperiod as a function of latitude also vary in diapause duration. In this species, the southern forms enter diapause in the fourth larval instar while northern populations enter diapause in the third instar (Bradshaw and Lounibos, 1977). Latitudinal variation in diapause duration also has been observed in *Heliothis zea* (Holtzer et al., 1976), *Chrysopa carnea* (Tauber and Tauber, 1972), *Acronycta rumicis* (Danilevskii, 1965) and *Ostrinia nubilalis* (McLeod, 1978).

In the case of *A. rumicis* and *O. nubilalis*, the more northern strains showed both a longer critical photoperiod and a longer diapause. Conversely, southern strains displayed a shorter critical photoperiod and a shorter diapause. The correlation of these two traits in these strains suggests that the same genetic mechanisms underlie both features
of diapause. This possibility could be tested by selecting strains for an alteration in either trait and observing the effect of selection upon the other trait.

A pleiotropic effect of this kind may be adaptively important in order to maximize the fitness of a population living in a given location. For example, in a northern population, early entrance into diapause in the fall and late emergence in the spring are both necessary to insure survival. If the same genetic factors influence both traits, then the nonadaptive consequences of early entrance and early emergence, or late entrance and late emergence, would be avoided. The consequences would be similar if the loci affecting capability and duration of diapause were closely linked. Differences in diapause duration can also act to isolate different strains temporally and serve as the basis for speciation (McLeod, 1978).

3. Interpretation of inheritance studies with natural strains

Because genetic variability in any number of features can affect diapause incidence and because genetic-environmental interactions tend to be very large, patterns of inheritance may be difficult to interpret. Vinogradova and Bogdanova (1981) found that in a controlled environment, strains of *Calliphora vicina* and *Boettcherisca septentrionalis* show endogenous cycles of diapause incidence over a period of several generations. These cycles may reflect features of the inheritance pattern of diapause that are not readily analyzable because of the great complexity of interactions between environmental and genetic factors.
Interstrain crosses can never reveal the mode of inheritance or the underlying genetic control of any trait, particularly one as complex as diapause capability. Any number of genotypes can give rise to an observed level of diapause and the effects of individual loci, even in the absence of complicating interactions, cannot always be separated easily. Genetic and environmental interactions surely affect the expression of gene loci that regulate various aspects and diapause and in the process further complicate interpretation. Because natural populations adapt to local selective forces, the diapause traits of strains derived from different locales tend to show a high degree of polymorphism (Tauber and Tauber, 1981). These differences often reflect a high level of genotypic diversity that is not easily evaluated by interstrain crosses. Rabb's (1969) inconclusive results with pupal diapause in Manduca sexta may arise from the fact that one strain studied was subtropical and the other, temperate, in origin. The complexity of results from the interstrain matings may reflect a high degree of polymorphism. At the other extreme, the effect of loci that play a role in diapause regulation may go undetected because the loci are homozygous in the studied strain.

C. **Selection Studies**

The rapid response of lines selected for diapause traits indicate that much of the variability for diapause response arises from genetic differences.

Perhaps because diapause is often an undesirable trait for laboratory rearing purposes, numerous studies anecdotally report
reduction of a diapause response as a consequence of selection. Selection for low diapause incidence has been accomplished in strains of *Diabrotica virgifera* (Branson, 1976), *Christoneura fumiferana* (Harvey, 1957), *Melanoplus sanguinipes* (Pickford and Randell, 1969), *Gryllus campestris* (Ismail and Fuzeau-Braesch, 1972), and *Hyalophora cecropia* (Waldbauer and Sternberg, 1973). Among the Diptera similar results have been obtained in *Rhagoletis pomonella* (Baerwald and Boush, 1967), *Pseudosarcophaga affinis* (House, 1967), *Poecilometopa spilogaster* (Denlinger, 1979), and *Drosophila littoralis* (Lumme and Pohjola, 1980). In none of these studies was a genetic analysis performed, but the relatively rapid responses and the stability of the resulting lines strongly indicate the importance of genetic factors. A most striking example of selection for nondiapause occurs in *Lymantria dispar* (Hoy, 1977). In eight generations of selection, a nondiapause strain was derived from a strain that originally was almost completely univoltine (and therefore underwent a larval diapause in every generation).

Less common are examples of selection for high diapause incidence, presumably because such lines take longer to develop. In *Heliothis zea*, lines for both low (Herzog and Phillips, 1974) and high diapause incidence (Herzog, 1976) have been obtained although studies of crosses between these lines have not been done. A high diapause line has been produced by selection in *Calliphora vicina* (Vinogradova and Tsutskova, 1976) and *Lucilia caesar* (Ring, 1971).
Because diapause involves environmental components, the analysis of selection lines must be done carefully. Most of the studies described here utilize a continuously controlled environment upon each generation and individuals are selected on the basis of the desired diapause phenotype. The inheritance of diapause in Bombyx, however, serves to illustrate the problems which must be addressed when interpreting selection data. If one were to perform a selection for nondiapause in a short day regime on consecutive generations of Bombyx the results would indicate a rapid response to selection, implying a few simple genetic differences. Actually, the mechanism responsible for this reduction is physiological. Pre-adult environmental effects have not been rigorously analyzed in most insect species.

D. Interstrain Crosses Involving One or More Selected Lines


Genetic analyses have been performed for many species utilizing strains that differ in diapause characteristics. Many times the dependability of diapause parameters has not been tested by utilizing replications and/or control groups and therefore the reliability of the results is questionable. Furthermore, the origins of the strains studied are geographically and/or temporally diverse, further complicating interpretation of the results. Typically, genetic studies of diapause involve crosses between naturally derived strains and strains selected for nondiapause.
A cross of this type in *Anthonomus grandis*, the cotton boll weevil, involved a natural strain showing an incidence of 40.4% and a selected strain with an incidence of 2.03% (McCoy et al., 1968). The $F_1$ progeny tended to show a low diapause incidence and backcrosses showed similar patterns, indicating that the low diapause trait was dominant. The diapause incidence of the $F_2$ reciprocal crosses ($\text{Low and High-} F_2 \text{ vs. } \text{High x Low-} F_2$) were significantly different possibly as a consequence of maternal effects. The results of these crosses indicated that the genetic differences might involve primarily the effects of a single autosomal locus.

A strain of the false melon beetle, *Atrachya menetriesi*, has been successfully selected for low diapause. Crosses between this strain and a strain showing a higher incidence of diapause resulted in eggs whose diapause capability depended upon the female parent (Ando and Miya, 1968). A male influence appeared in $F_2$ eggs, a reflection perhaps of the fact that half the $F_1$ female's genome originates in the male. The diapause ratios among $F_2$ and $F_3$ progeny did not correspond to any simple genetic models.

The most complete analysis of the diapause inheritance in selection lines has been performed in the two-spotted spider mite, *Tetranychus urticae* (Helle, 1968). In these experiments, the genetic differences were analyzed by crossing inbred lines derived from the same original line. After seven generations of inbreeding, lines that by chance showed the longest and shortest critical photoperiod were crossed because they would most likely show the effects of one or a
few major gene loci. The high response (long critical photoperiod) was incompletely dominant among the $F_1$ progeny of these crosses. Reciprocal $F_1$ - backcrosses to low response males ($(High \times Low) \times Low$ and $(Low \times High) \times Low$) indicated that the transmission is different between the two crosses. Helle suggests that the differences arise from a cytoplasmic determinant or as a consequence of sex-determined differences in diapause capability.

2. Selection and analysis for diapause duration

Strains with altered lengths of diapause, often referred to as diapause intensity, have also been developed and occasionally analyzed genetically.

Strains for long and short diapause duration have been successfully developed in *Pectinophora gossypiella*, the pink bollworm (Langston and Watson, 1975) as well as strains for high and low diapause incidence (Barry and Adkisson, 1966; Bartlett, 1977). Interstrain crosses reveal that sex-linkage or maternal factors influence diapause duration, but $F_2$ and backcrosses were not made, limiting the possibilities of interpretation. Unfortunately, no studies have analyzed the possibility of genetic relationship between diapause incidence and duration in this species.

As in the case of natural strains, diapause incidence and duration are not unrelated traits in *Atrachya menetriesis* (Ando and Miya, 1968). Males selected for low diapause were mated with females destined to produce diapausing eggs. The eggs from these crosses show an unstable,
short diapause relative to the eggs from crosses of individuals from the original strain.

3. Interpretation of inheritance studies with selection lines

Most of the problems encountered in the study of natural strains also affect the reliability of results in selected strain studies. The most acute limitation is the diverse origin of the strains used for analysis. The approach taken by Helle (1968) with strains derived from an original stock provides the best method for analyzing the genetic differences that lead to differences in photoperiodic response.

E. Mutant Studies

While abundant evidence suggests that genetic factors affect diapause, almost no work discusses how genetic expression affects diapause induction and maintenance. The most concise approach to answer these questions is genetic dissection, the isolation and study of mutants that affect diapause.

Only in *Tetranychus urticae* (Veerman, 1980) have diapause mutants been isolated and studied. Females of this species that are homozygous for a mutation which disrupts \( \beta \)-carotene synthesis also show a decreased ability to enter diapause. However, if the mother is heterozygous for the mutation, her progeny will respond to photoperiod, presumably because the mother transmits enough pigment to allow a photoperiodic response. With this qualification, the inheritance of the trait follows a pattern of inheritance consistent with an allelic difference at a single locus.
Drosophila melanogaster does not enter diapause at any stage of the life cycle, but the isolation of conditional mutants that show no ecdysone (Garen et al., 1977) and juvenile hormone activity (Arking and Vlach, 1976) has been accomplished. Similar mutants in a diapausing species would present a tremendous tool for studies of genetic and neuroendocrine regulation of diapause processes.

The importance of gene expression in diapause induction and maintenance has been established in biochemical studies of Celerio euphorbiae (Grzelak et al., 1974). Acetylcholinesterase activity at the end of diapause depends upon renewed transcription and translation of DNA. Exogenous applications of JH, a hormone known to play a regulatory role in diapause, can prematurely enhance transcription of RNA in diapausing pupae (Grzelak et al., 1975, 1981). These findings indicate that gene expression itself is shut down in the diapause state. Diapause induction, therefore, can be viewed as a process that leads to an absence of developmental gene expression.

The finding that embryonic diapause in B. mori results from the presence of a neuropeptide suggests that gene expression might be directly altered by the photoperiodic environment. This fascinating possibility is further enhanced by the discovery of circadian rhythm mutants in D. melanogaster (Knopka and Benzer, 1971). Photoperiodic response and circadian rhythms have been functionally linked in several studies of S. argyrostroma (Saunders, 1971; 1973).
F. Objectives of the Study

Many anecdotal accounts in the literature suggest that diapause traits have a genetic basis. Few studies have actually involved controlled, interline crosses and only once (Helle, 1968) have interline crosses involved descendents from a single original strain.

My study with the flesh fly, *Sarcophaga bullata*, attempts to develop through artificial selection, lines that show a high and low incidence of pupal diapause in a diapause-inducing environment. Reciprocal parental, $F_1$, and backcrosses are performed and the progeny analyzed for diapause incidence. These results can determine to what extent genetic differences correlate with differences in response.

Problems encountered in the direct selection for high diapause incidence also led to new lines of inquiry focusing on (1) development of a high incidence line by indirect selection, and (2) a description of the underlying causes for the failure of direct selection. The results of these studies lead to important inferences about the genetic basis of the diapause response.

G. Justification for Study of *S. bullata*

The flesh fly is a particularly amenable species for the genetic study of diapause because (1) its life cycle is relatively short and females produce large numbers of progeny, and (2) an overview of the environmental, physiological, and morphological features of pupal diapause in *Sarcophaga* has been attained. Furthermore, a chromosome
map already exists for five of the six linkage groups in the species (Whitten, 1969). The sixth group is actually a microsome.

H. Life Cycle and Diapause Characteristics

The life cycle of S. bullata follows a pattern fairly typical of other cyclorrhaphous Diptera. The rate of development in every stage of the life cycle is temperature dependent and the following discussion is true only for individuals reared at 25°C.

The adult fly requires a protein meal for egg maturation (Pappas and Fraenkel, 1977) and mates a few days after eclosion. The embryo develops in the mother's uterus during the next five days and first instar larvae are deposited on flesh or fecal material at this time. Two larval molts ensue, followed by a wandering larval period that lasts 24 to 48 hours. Pupariation occurs at this time and is easily detectable by a tanning of the cuticle. Pupation occurs about two days later. The life cycle is completed approximately 20 days after pupation, when the adult ecloses.

The fly, in response to short daylength during the embryonic and early stages, can enter a pupal diapause shortly after pupation (Denlinger, 1971). The embryonic response to short days is not mediated by the mother (Denlinger, 1971) and pupal diapause becomes increasingly likely as larval temperature is reduced.

The induction of diapause apparently involves a clock mechanism (Saunders, 1971, 1973) that measures night length (Gnagey, 1981). Very few studies have investigated the biochemical aspects of diapause.
"programming", although levels of cyclic guanosine monophosphate (cGMP) are higher in long day embryos than in short day embryos (Gnagey, 1981).

Physiologically, exposure to short days results in a lack of prothoracicotropin hormone (PTTH) production in the neurosecretory cells (NSC). PTTH is necessary for stimulation of the prothoracic gland, the site of ecdysone synthesis. In the absence of PTTH, the prothoracic gland remains inactive (Zdarek and Fraenkel, 1971). Whether destined for diapause or not, all larvae experience a burst of ecdysone activity that leads to pupariation, but nondiapausing pupae also experience a second burst of activity about three days later (Shaaya and Karlson, 1965; Walker and Denlinger, 1980). This burst causes the differentiation of imaginal discs into adult tissue. By contrast, diapausing pupae lack the second burst (Ohtaki and Takahashi, 1972; Walker and Denlinger, 1980) and remain in an undifferentiated state. As the pupa enters diapause oxygen consumption drops and remains considerably lower than in a nondiapause state, although periodic fluctuations are detectable (Denlinger et al., 1972). In addition, cycles of juvenile hormone (JH) activity are found in diapause destined pupae although the role of JH remains unknown.

At the termination of diapause, titers of ecdysone increase dramatically and remain high for several days (Walker and Denlinger, 1980). This sustained level of ecdysone stimulates adult differentiation (Denlinger, 1981a).

The importance of gene activity in the entry into diapause can be appreciated by considering that both ecdysone and juvenile hormone have
been implicated in gene expression (Truman and Riddiford, 1978). No work to date has investigated the genetic parameters associated with the diapause response in Sarcophaga.
Chapter II
CROSSES INVOLVING LINES SELECTED FOR HIGH AND LOW DIAPAUSE INCIDENCE

A. Introduction

The ability to enter diapause is environmentally regulated but genetically determined. Insect strains isolated from natural populations show a photoperiodic response that correlates with geographical origin (Bradshaw, 1976). Furthermore, crosses between members of naturally derived strains reveal patterns of inheritance that indicate a high proportion of genetic variability (Danilevskii, 1965; Lumme et al., 1975; Hoy, 1978) although modes of inheritance for photoperiodic response vary widely between species.

Inheritance studies with natural strains suffer because the genetic variability obtained cannot be controlled. The diapause traits of strains chosen for study show varying degrees of polymorphism that reflect underlying genotypic differences. In conjunction with the genetic and environmental interactions that affect gene expression at loci that influence diapause, these genetic differences are not easily evaluated.

Two-way artificial selection and subsequent inbreeding provide a way to maximize the genetic differences between the two lines and to increase the likelihood of homozygosity at those loci that affect
diapause. By crossing inbred strains for high and low diapause incidence selected from the same original strain, the genetic differences that give rise to these different levels of response can be elucidated and studied. Helle (1968) developed lines of *Tetranychus urticae* in this way for differences in photoperiodic response. This allowed him to correlate differences in response with gene differences.

In this study, a similar approach is taken. Lines of *Sarcophaga bullata* have been developed for high and low diapause incidence. With interstrain, F\(_1\), and backcrosses, any gene differences that underlie the strain differences in diapause response can be ascertained and evaluated.

B. Materials and Methods

The selected lines were derived from a strain of *S. bullata* that originated in Lexington, Massachusetts in 1974. At 12L:12D, (light: dark cycle) and 18\(^\circ\)C, the strains showed a diapause incidence of 100% (Denlinger, unpublished). At 12L:12D, 25\(^\circ\)C, no pupae entered diapause. Before the selection process was initiated the strain showed an incidence of 47.0% at 12L:12D, 23\(^\circ\)C and had been sustained in a nondiapause inducing environment (15L:9D, 25\(^\circ\)C) prior to the study.

The procedures for rearing the flies were based upon those developed by Denlinger (1972b). Developing pupae were contained in cylindrical, one quarter plastic containers half-filled with sawdust. These containers were placed in screen cages (12" x 12" x 12") several days before eclosion. At the time of adult eclosion, a water dish
and several sugar cubes were placed in the cage, and these were replenished until the collection of larvae. Liver was utilized as a source of protein and placed in the cage on the second day. The piece of liver was replaced daily until Day 6. For the remainder of the time until larvae collection, dried milk was fed as a source of protein. Approximately 80 flies were kept in a single cage and these were allowed to mate and feed freely. Larvae were collected on Day 11 by placing a piece of liver in the cage for the females to use as a larviposition site. In those instances when progeny were drawn from individual females, larvae were extruded from the gravid mother by gently squeezing her abdomen.

Approximately 80 larvae were then transferred to a piece of liver weighing approximately 40 gms. This later was wrapped in an aluminum foil packet and placed in a plastic container (described above) half-filled with sawdust. The larvae fed until late in the third instar, left the liver packet, and then burrowed into the sawdust. After all larvae were done feeding, the liver packet was removed to prevent a buildup of excess moisture in the container.

Adult members of the original strain were mass mated and reared in a 12L:12D (light:dark cycle), 25°C regime and females were allowed to larviposit on the eleventh day after eclosion. Larvae were transferred to 12L:12D, 23°C. Approximately thirty days after larviposition, diapausing pupae were detected by the absence of differentiating antennal imaginal discs (Fraenkel and Hsiao, 1968). Twenty individuals that had not entered diapause were randomly
selected and mated after adult eclosion. The progeny were reared in the same way and the selection procedure repeated for three more generations.

In the fifth generation, larvae were placed at 12L:12D, 20°, a regime that induces a high level of diapause. This procedure was followed through the eleventh generation. The larvae reared in the eleventh, twelfth, and thirteenth generations originated from a single female in order to maximize the homozygosity of all gene loci including those that affect the diapause response. In the twelfth generation, all larvae were reared at 15L:9D, 25° to eliminate the possibility of a maternally-induced reduction in diapause as a consequence of larval exposure to short days (see Chapter IV). The sibling individuals that comprised the thirteenth generation were utilized for the genetic study as the low diapause line (L).

Direct selection for a line showing a high incidence of diapause was unsuccessful and later shown to be impossible (Chapter IV). Therefore, selection for a high diapause line (H) was accomplished indirectly, by selecting individuals that purpariated relatively late.

The selection data were obtained for the low line, but has not been included for consideration for the following reasons: (1) environmental problems encountered at various times during the selection procedure limits the reliability of the data; (2) the environment utilized for selection was changed during the experiment; (3) the data for early generations come from randomly drawn samples, whereas later data are based on the progeny of individual females; (4) subsequent findings indicate that the rearing of consecutive generations
at 12L:12D does not allow for a true indication of selection response (Chapter IV); and (5) no data exist for the line selected for high incidence until after selection was completed.

Interline crosses were made by mass mating males of one line and females of the other at 12L:12D, 25°C. The larvae were taken from individual females and placed at 12L:12D, 20°C. This procedure produced data for two crosses (females designated on left side): H x L and L x H.

Because of the maternal effect, hybrids for F₁ crosses and backcrosses, as well as members of the H and L lines, were also mated in long day conditions (15L:9D, 25°C). The progeny of these crosses were mated in 12L:12D, 25°C to produce data for the following groups: H x (HxL), H x (LxH), (HxL) x H, (LxH) x H, L x (HxL), L x (LxH), (HxL) x L, (LxH) x L, (HxL) x (HxL), and (LxH) x (LxH). Progeny of these crosses were placed in 12L:12D, 20°C after larviposition and later scored for diapause.

C. Results

Table 1 shows the diapause incidence of the progeny from all the crosses. These figures are pooled samples from individual females (Appendix A). The range of percentages fell between 30% and 70% and therefore the arcsin transformation was not used (Sokal and Rohlf, 1969).
Table 1. Pupal diapause incidence at 12L:12D, 20°C of parental, F₁, and backcrosses using members of line of *S. bullata* selected for high diapause incidence (H) and low diapause incidence (L).

<table>
<thead>
<tr>
<th>Cross (♀ x ♂)</th>
<th>No. of Females</th>
<th>No. of Larvae</th>
<th>Diapause Incidence (%)</th>
<th>Standard Deviation (%)</th>
<th>Expected Value (Additive Model)</th>
<th>Deviation (d)</th>
<th>Variance of d (V₃d)</th>
<th>Test Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>H x H</td>
<td>6</td>
<td>207</td>
<td>92.7</td>
<td>3.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L x L</td>
<td>5</td>
<td>220</td>
<td>24.5</td>
<td>8.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H x L</td>
<td>3</td>
<td>149</td>
<td>42.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L x H</td>
<td>3</td>
<td>58</td>
<td>44.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F₁ (Cum.)</td>
<td>6</td>
<td>207</td>
<td>42.9</td>
<td>11.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(H x L) x (H x L)</td>
<td>9</td>
<td>409</td>
<td>33.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(L x H) x (L x H)</td>
<td>9</td>
<td>630</td>
<td>47.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F₂ (Cum.)</td>
<td>18</td>
<td>1039</td>
<td>42.3</td>
<td>2.3</td>
<td>50.8 %</td>
<td>0.3</td>
<td>9.6 x 10⁻³</td>
<td>3.45**</td>
</tr>
<tr>
<td>H x (H x L)</td>
<td>9</td>
<td>558</td>
<td>80.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H x (L x H)</td>
<td>7</td>
<td>353</td>
<td>70.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(H x L) x H</td>
<td>5</td>
<td>150</td>
<td>65.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(L x H) x L</td>
<td>4</td>
<td>263</td>
<td>68.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCH (Cum.)</td>
<td>25</td>
<td>1324</td>
<td>73.6</td>
<td>1.4</td>
<td>67.8 %</td>
<td>0.1</td>
<td>2.1 x 10⁻³</td>
<td>2.52*</td>
</tr>
<tr>
<td>L x (H x L)</td>
<td>11</td>
<td>689</td>
<td>33.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L x (L x H)</td>
<td>5</td>
<td>250</td>
<td>24.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(H x L) x L</td>
<td>2</td>
<td>96</td>
<td>36.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(L x H) x L</td>
<td>6</td>
<td>458</td>
<td>21.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCL (Cum.)</td>
<td>24</td>
<td>1493</td>
<td>28.5</td>
<td>1.3</td>
<td>33.7 %</td>
<td>0.1</td>
<td>5.3 x 10⁻³</td>
<td>1.43 n.s.</td>
</tr>
</tbody>
</table>

* P(Z) < 0.05  
** P(Z) < 0.01  
n.s.: not significant
The simplest genetic model by which to test these results assumes additive inheritance with no dominance. With no dominance the $F_1$ progeny should show a diapause incidence intermediate between the two parental lines (58.6%). The actual pooled $F_1$ incidence (43.0%) varies significantly from the results expected with no dominance ($P < 0.01$, Z-test). Therefore, the hypothesis that the inheritance of diapause among these lines is additive and the low trait is incompletely dominant was tested.

Large differences existed within the $F_1$, $F_2$, and backcross groups (although significant differences occur between them) and therefore the data were consolidated into four groups for the purpose of testing the additive hypothesis: (1) $F_1$ progeny, (2) $F_2$ progeny, (3) backcrosses to the high line, and (4) backcrosses to the low line.

The probability is $D$ that a larva will enter diapause when endowed with a particular genotype and the distribution of all samples of larvae is binomial with a mean equal to $D$ and a variance $D(1 - D)/N$, where $N$ is the sample size. Using this rationale a variance was calculated for the backcrosses, the $F_1$, and $F_2$ progeny (Table 1). The standard deviation was derived by taking the square root.

The expected diapause incidence ($E$) for an additive model in the backcross (BCH and BCL) and $F_2$ data were generated with the following equations. $D$ indicates the observed diapause incidence:

$$E_{F_2} = \frac{D_H}{4} + \frac{D_F}{2} + \frac{D_L}{4}$$

$$E_{BCH} = \frac{D_H + D_F}{2}$$
If the observed and expected values in the \( F_2 \) group are equal, then

\[ E_{F_2} = D_{F_2} \quad \text{and} \quad D_{F_2} = D_H/4 - D_{F_1}/2 - D_L/4 = 0. \]

By algebraic manipulation,

\[ 4D_{F_2} - D_H - 2D_{F_1} = 0. \]

Using the actual results, the deviation, \( d \), from an additive model can be calculated for the \( F_2 \). The same line of reasoning can be applied to the backcrosses. Therefore:

\[
4D_{F_2} - D_H - 2D_{F_1} - D_L = d_{F_2}
\]

\[
2D_{BCH} - D_H - D_{F_1} = d_{BCH}
\]

\[
2D_{BCL} - D_L - D_{F_1} = d_{BCL}
\]

The variance of the deviation, \( V_d \), can also be calculated:

\[
V_d_{F_2} = 16V_{F_2} + V_H + 4V_{F_1} + V_L
\]

\[
V_d_{BCH} = 4V_{BCH} + V_H + V_{F_1}
\]

\[
V_d_{BCL} = 4V_{BCL} + V_L + V_{F_1}
\]

The resulting test statistic, \( d/\sqrt{V_d} \), is normally distributed and therefore a Z-test was applied to the data. The results of this test show that the BCL incidence fits an additive model. By contrast, the \( F_2 \) \((P < 0.01)\) and BCH \((P < 0.05)\), do not fit an additive model, indicating that genetic and genetic-environmental interactions are involved.
In Figure 1, a comparison is made between the actual data and the data predicted by an additive model with incomplete dominance of the low diapause trait. The results show that genetic factors clearly play an important role in the capability for diapause in flesh flies.

D. Discussion

The results suggest that the gene differences associated with differences in diapause capability in the two studied strains of S. bullata may be multiple and may involve genetic-environmental complex interactions. These results coincide with other similar studies (Hoy, 1978) and are not surprising in view of the complex nature of the diapause phenotype. While many studies have investigated the importance of environmental factors that influence the diapause response (Denlinger, 1972a; 1972b; Saunders, 1971; 1973) this study presents the first strong evidence that genetic factors greatly influence diapause capability in S. bullata.

Among environmental studies of diapause in Sarcophaga, the importance of several hormones has been demonstrated (Ohtaki and Takahashi, 1972; Zdarek and Denlinger, 1975; Walker and Denlinger, 1980; Denlinger, 1981a). In addition, embryonic levels of cyclic GMP and various clock mechanisms also affect diapause capability (Gnagey, 1981; Saunders, 1971, 1973). Genetically based differences that alter any of these mechanisms presumably can also change the ability to enter diapause.
Figure 1. A comparison of observed diapause incidence at 12L:12D 20° among progeny of crosses involving lines of *S. bullata* selected for high (H) and low (L) diapause incidence with results predicted by an additive model with incomplete dominance.
Further insight into the genetical control of developmental and biochemical events associated with diapause can be gained through the isolation and analysis of mutants that affect diapause. Pigmentation mutants that reduce diapause have already been isolated and studied in *Tetranychus urticae* (Veerman, 1980) and the flesh fly appears to be a suitable candidate for genetic dissection techniques of this type.
Chapter III

EFFECTS OF SELECTION FOR LATE PUPARIATION
ON DIAPAUSE INCIDENCE AND DURATION

A. Introduction

A variety of insect studies suggest that diapause incidence and duration are highly heritable (Hoy, 1978; Tauber and Tauber, 1981). Several experiments with natural populations suggest that diapause incidence and duration are not independently controlled traits. Bivoltine and univoltine strains of Ostrinia nubilalis show differences in both incidence and duration of diapause in controlled environmental conditions (MacLeod, 1978) and hybrids show characteristics for both traits which resemble those of the univoltine strain. Strains derived from northern populations of Acronycta rumicis enter diapause at a longer critical daylength and remain in diapause longer than strains isolated from more southern clines (Danilevskii, 1965). These observations lead to the inference that the same genetic factors which alter the capability for diapause also affect diapause duration.

In these experiments I test the association between pre-diapause developmental time, diapause incidence, and diapause duration using the flesh fly, Sarcophaga bullata. This species, like other temperate zone flesh flies (Denlinger, 1981a) relies on short daylength
and cool temperature to provide the primary signals for induction of pupal diapause (Denlinger, 1972a, 1972b). Larvae destined for diapause tend to pupariate later under controlled conditions than those not entering diapause (Saunders, 1971, 1973; Denlinger 1972a). Even in nondiapausing conditions, duration of larval development shows some variability. In the present study I use an artificially selected strain to test the hypothesis that the observed variability in the larval developmental rate is genetically based and that genetic factors influencing developmental rate also affect diapause incidence and duration.

B. Materials and Methods

Rearing procedures and the strain studied were described in Chapter II (Denlinger, 1972a).

Approximately 600 larvae were reared together in a nondiapause environment (15L:9D, 25°C) and the last 25 larvae that pupariated were selected and raised to adulthood. These adults were mass mated and the selection procedure repeated for six successive generations. Another strain was reared for seven generations in the same environment but members were not selected on the basis of any phenotype.

New adults of each line were then transferred to 12L:12D, 25°C and the progeny of individual females were transferred to 12L:12D, 20°C (strongly diapause-inducing conditions) and maintained in these conditions through the onset of pupal diapause. The incidence of diapause was determined 35-40 days after larviposition according to the criteria established by Fraenkel and Hsiao (1968). Pupae from both
groups were transferred to 25°C 60 days after larviposition, and the time of diapause termination was recorded.

C. Results

As a consequence of six cycles of selection for late pupariation at 15L:9D, 25°C, the selected line showed a significantly greater variance (F-test, P < 0.05) and pupariated significantly later than the unselected line (P < 0.01, T-test) in long day conditions (Table 2). The median and modal length of the larval stage was about five days in both groups. The difference in mean duration of the larval stage arose primarily because more individuals in the selected line pupariated 6 or more days after larviposition (Figure 2).

Selection for a longer larval stage also increased the duration of the other stages of the life cycle. The average life cycle in the selected line was 3.8 days longer than the mean life cycle of the unselected line. This difference cannot be fully accounted for by the difference in the duration of the larval stage.

In diapause inducing conditions (12:12, 20°C), the larval stage lasts considerably longer in both lines because larval developmental rate is temperature dependent (Figure 2). Mean duration of the larval stage in the late selected line is about one day longer than in the unselected group (Table 2) and again, the variance of the selected line (F-test, P < 0.05) is significantly greater than the variance of the original line. Likewise, the median (day 23) and mode (day 22) of the selected line was later than in the unselected line (day 21 for both median and mode). Though differences in mean duration of the larval
Table 2. Comparison of length of larval stage, length of life cycle, diapause incidence, and diapause duration between an original strain of S. bullata and a strain selected for late pupariation. Number of individuals in parentheses and standard deviation indicated after mean values.

<table>
<thead>
<tr>
<th></th>
<th>Unselected</th>
<th>Selected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean duration of larval stage at 15L:9D, 25°</td>
<td>5.5 ± 0.6 d (219)</td>
<td>6.3 ± 0.7 d (232)</td>
</tr>
<tr>
<td>Mean duration of life cycle at 15L:9D, 25°</td>
<td>26.7 d</td>
<td>30.5 d</td>
</tr>
<tr>
<td>Mean duration of larval stage at 12L:12D, 20°</td>
<td>21.5 ± 1.6 d (203)</td>
<td>22.5 ± 2.4 d (216)</td>
</tr>
<tr>
<td>Diapause incidence (%) at 12L:12D, 20°</td>
<td>63.9% (202)</td>
<td>95.6% (182)</td>
</tr>
<tr>
<td>Mean duration of diapause after transfer to 25°C, 60 days after larviposition</td>
<td>8.73 ± 7.5 d (82)</td>
<td>34.7 ± 9.0 d (70)</td>
</tr>
</tbody>
</table>
Figure 2. Time of pupariation for an unselected and selected strain of *S. bullata* at 15L:9D, 25°C and 12L:12D, 20°C.
stage are not large, they are statistically significant ($P < 0.01$, T-test) and give rise to very large differences in diapause incidence (Table 2). The diapause incidence of the selected line was the highest recorded for any line derived from our S. bullata colony.

Substantial differences also exist between the lines in the amount of time that elapses before initiation of pharate adult development (Table 2). When transferred to 25°C, most individuals in the unselected line begin to develop within ten days while in the selected group, pupae break diapause much later and over a longer period of time (Figure 3).

D. Discussion

The experiments show that artificial selection for greater duration of the larval stage in nondiapausing conditions produced a population that tends to pupariate later in both nondiapause and diapause-inducing conditions. Those genetic factors that affect larval developmental rate also affect developmental rate in other stages of the life cycle. Moreover, these individuals display a greater likelihood to enter diapause and remain in diapause considerably longer than unselected individuals. Therefore, those genetic factors that affect developmental rate probably influence diapause parameters.

A viable alternative explanation for the results is that those gene loci that affect developmental rate are closely linked to loci affecting diapause. While separate loci may account for the relationship, linkage would nevertheless tend to associate the two traits and maintain the adaptive advantages of keeping the two traits together.
Figure 3. Termination of diapause when pupae of an unselected and selected strain of *S. bullata* were transferred from 20° to 25°C 60 days after larviposition.
The higher variance of the selected group relative to the unselected group contradicts the expectation that variance decreases as a consequence of selection. Selection normally decreases phenotypic variability by decreasing heterozygosity and genetic variability. However, if the frequency of alleles that tend to lengthen pupariation initially was very low, then selection for late pupariation might increase heterozygosity at those loci and increase the variance. This explanation also indicates that selection for still later pupariation is possible in the selected lines.

At present, we cannot be certain how developmental rate might influence the response to diapause. Denlinger (1981a) suggests that the embryonic programming for diapause also affects larval developmental rate and thus accounts for the delay in pupariation seen among larvae destined for diapause. Following this line of reasoning, individuals that tend to pupariate later might actually be more likely to diapause because the same genetic factors affect both diapause programming and developmental rate. In contrast, the photoinduction model of Saunders (1971, 1973) explains the greater likelihood to enter diapause among late pupariating individuals as a consequence of exposure to more photoperiodic cycles. Therefore, those genetic factors that influence developmental rate would influence diapause capability indirectly in an appropriate environment. The present results cannot reconcile the issue but they do represent the first experimental evidence that developmental rate and diapause induction and maintenance are components of the same system in *S. bullata*. These common elements are genetically based.
Termination of diapause in *Sarcophaga* is a two-part process: completion of a temperature-insensitive phase is followed by a temperature sensitive phase in which the pupae respond immediately to high temperature. The observation that most unselected individuals break diapause within ten days after transfer to 25°C suggests that the insensitive phase is already over for most members of this group. Delay in resumption of development in the late line may occur because these individuals still remain in the insensitive stage.

The relationship between diapause induction and duration may serve an adaptive role in nature. Northern populations usually enter diapause earlier in the season and must remain in diapause longer. By maintaining both parameters with common genetic elements, the appropriate diapause phenology could be readily sustained within a population.

The observed genetic variance is likely to reflect interesting differences in the neuroendocrine events that regulate diapause. Prothoracicotropic hormone, ecdysone, and juvenile hormone have all been implicated in the diapause regulatory scheme of *Sarcophaga* (Fraenkel and Hsiao, 1968; Ohtaki and Takahashi, 1972; Zdarek and Denlinger, 1975; Gibbs, 1976; Walker and Denlinger, 1980; Denlinger, 1981b), and future studies with genetic variants should prove useful for identifying the contribution of the various hormones.
Chapter IV
AN ENVIRONMENTALLY REGULATED MATERNAL EFFECT THAT ALTERS DIAPAUSE IN THE NEXT GENERATION

A. Introduction

Among flesh flies of the genus Sarcophaga exposure of embryos and larvae to short days results in the capability for subsequent pupal diapause (Denlinger, 1971; Saunders, 1971; Ohtaki and Takahashi, 1972; Vinogradova, 1976). Though the embryo resides within the uterus of its mother, the mother does not mediate or process the photoperiodic cues during embryogenesis. Instead, the embryo receives and responds to the stimuli directly (Denlinger, 1971). In all these previous experiments with pupal diapause the parental generations were reared under long day conditions prior to adult eclosion. Consequently, the adults had no history of diapause. I now find that the results are dramatically different if, instead, the flies have a history of pupal diapause. When flies that had previously been in diapause are mated and their offspring reared in a strongly diapause inducing environment, virtually none of the progeny enter pupal diapause. In the present study I define the events leading to elimination of diapause and evaluate the roles of both the male and female parents in transmitting this effect. Furthermore, I investigate what, if any, effects exogenous juvenile
hormone and ecdysone may exert on expression of the maternal effect. Both of these hormones have already been implicated as regulators of pupal diapause in Sarcophaga (Fraenkel and Hsiao, 1968; Ohtaki and Takahashi, 1972; Zdarek and Denlinger, 1975; Walker and Denlinger, 1980; Denlinger, 1981b) and recent experiments by Briers and de Loof (1980) indicate that ecdysone is indeed present in adult females of S. bullata.

B. Materials and Methods

The origin of the Sarcophaga bullata colony and the rearing procedures have been described. A strain of S. crassipalpis isolated in Illinois (Denlinger, 1972b) was used in one experiment. The technique for in vitro culture of embryos was described previously (Denlinger, 1971). Embryos were squeezed from gravid females onto a piece of moistened filter paper placed inside a Petri dish. Each dish contained embryos from a single female and was sealed with a strip of wax paper to retain moisture. Until they hatched as larvae, the embryos were raised at 12L:12D, 25°C. After hatching, the larvae from each dish were transferred to 12L:12D, 20°C and reared according to the procedures described earlier.

Flies with no diapause history were raised before eclosion at 15L:9D, 25°C (nondiapause conditions) unless otherwise indicated. Flies with a diapause history were produced by exposing them as embryos to 12L:12D and 25°C and then rearing them as larvae at 12L:12D, 20°C. Parents of flies with a diapause history had been reared at 15L:9D and 25°C until adult eclosion. To test the capacity of the flies for
pupal diapause I exposed the adult flies containing embryos in their uterus to 12L:12D, 25°C and after larviposition larvae were placed at 12L:12D, 20°C.

Diapause incidence was scored approximately 40 days after larviposition using the criteria established by Fraenkel and Hsiao (1968). To break diapause, pupae were transferred from 20°C to 25°C forty to sixty days after larviposition. The development of several flies was coordinated to permit synchronous adult eclosion by switching individual pharate adults between the two temperatures.

Both females with a diapause history and those with no diapause history were treated with exogenous applications of a juvenile hormone analog or 20-hydroxyecdysone. The progeny of these females were then counted for diapause.

For the 20-hydroxyecdysone treatments, females were injected on the dorsum of the thorax with 1 μl of hormone solution using a finely drawn 5 μl micropipette. After injection, the wound was sealed with hot wax and the females were mass mated to males from the same brood. Ecdysone solutions were prepared by dissolving 20-hydroxyecdysone (Sigma Chemical Co.) in de-ionized water. In this experiment the following treatment groups were tested: (1) no injections; (2) an injection of de-ionized water on the second and third day after eclosion; (3) 0.1 μg ecdysone solution on the second and third day after adult eclosion; and (4) 10 μg ecdysone on the second day after adult eclosion.
The juvenile hormone analog was applied with a 5 µl micropipette to the dorsal side of the abdomen. These solutions were prepared by dissolving a juvenile hormone analog (ZR515) in acetone. Females were given one of the following treatments: (1) no application; (2) application of acetone on the second and third day after adult eclosion; (3) 0.5 µg JH on the second and third day after eclosion; and (4) 5.0 µg JH on the second day after eclosion.

C. Results

1. Reciprocal cross matings

When individuals reared under long day conditions, and therefore, with no history of pupal diapause, were mated and the progeny reared in diapause-inducing conditions, a substantial proportion entered pupal diapause (Table 3). Single pair matings among individuals with a history of pupal diapause produced progeny that did not enter pupal diapause. Reciprocal crosses between a parent with no diapause history and one with a diapause history can determine whether only one or both parents contribute to the reduction in diapause among the progeny. All crosses involving a female with a diapause history showed no diapause in the next generation while reciprocal crosses indicated that the male parent exerts no reducing influence upon diapause. Reduction in diapause incidence is strictly a maternal effect. In addition to the results shown in Table 3, over 70 additional crosses (N > 2900 progeny) have consistently verified the elimination of diapause in the progeny of females having a diapause history.
Table 3. Pupal diapause incidence at 12L:12D, 20°C among the progeny of individuals with a diapause history (D) and individuals with no diapause history (N).

<table>
<thead>
<tr>
<th>Cross (♀ x ♂)</th>
<th>Replication</th>
<th>No. of Females</th>
<th>No. of Larvae</th>
<th>Diapause Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N x N</td>
<td>a.</td>
<td>5</td>
<td>265</td>
<td>70.6</td>
</tr>
<tr>
<td></td>
<td>b.</td>
<td>6</td>
<td>148</td>
<td>46.0</td>
</tr>
<tr>
<td>D x D</td>
<td>a.</td>
<td>3</td>
<td>148</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>b.</td>
<td>2</td>
<td>110</td>
<td>0</td>
</tr>
<tr>
<td>N x D</td>
<td>a.</td>
<td>4</td>
<td>231</td>
<td>80.9</td>
</tr>
<tr>
<td></td>
<td>b.</td>
<td>5</td>
<td>217</td>
<td>54.4</td>
</tr>
<tr>
<td>D x N</td>
<td>a.</td>
<td>4</td>
<td>231</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>b.</td>
<td>6</td>
<td>219</td>
<td>0</td>
</tr>
</tbody>
</table>
2. **Elimination of maternal influence during embryogenesis**

Direct interaction between mother and offspring can be eliminated by culturing the embryos in vitro. Eggs are fertilized during embryogenesis at the time of ovulation (five days after adult eclosion) and embryogenesis ends about five days later. Earlier studies using females with no diapause history have shown that diapause incidence does not change as a consequence of extrauterine culturing (Denlinger, 1971). The same technique was used to determine whether reduction in diapause is the consequence of a factor transmitted from the mother to her progeny during embryogenesis. The results of this experiment (Table 4) show that the mother does not influence diapause incidence among her progeny after ovulation. The effect was already expressed in embryos removed from the female immediately after ovulation. Consequently, the difference must arise from events occurring prior to ovulation, quite possibly during oogenesis, a time of extensive genetic activity.

3. **Cue for inducing the maternal effect**

The maternal effect may occur as a result of diapause per se or alternatively, exposure to the short days that are involved in programming diapause. The two possibilities can be experimentally separated. When *S. bullata* is reared in a strongly diapause-inducing regime of short days and 20°C, diapause can be averted by transferring the flies from 20°C to 25°C at or before pupariation.

Figure 4 illustrates a variety of environmental regimes to which groups of larvae were exposed. Upon adult emergence, flies were mated and diapause incidence was recorded for the progeny. When reared
Table 4. Incidence of pupal diapause in S. bullata when embryos (12L:12D, 25°C) were removed from the female at daily intervals after ovulation, cultured in vitro, and transferred as larvae to 12L:12D, 20°C.

<table>
<thead>
<tr>
<th>Time of Removal from Mother (Days after Ovulation)</th>
<th>Mothers with No Diapause History</th>
<th>Mothers with a Diapause History</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Pupae</td>
<td>Incidence (%)</td>
</tr>
<tr>
<td>0</td>
<td>29</td>
<td>69.0</td>
</tr>
<tr>
<td>1</td>
<td>126</td>
<td>54.6</td>
</tr>
<tr>
<td>2</td>
<td>81</td>
<td>49.4</td>
</tr>
<tr>
<td>3</td>
<td>91</td>
<td>58.2</td>
</tr>
<tr>
<td>4</td>
<td>95</td>
<td>51.6</td>
</tr>
<tr>
<td>5</td>
<td>2177</td>
<td>66.4</td>
</tr>
</tbody>
</table>
under short days and 20°C through the onset of diapause, 43.1% of the larvae entered diapause (Fig. 4A). As expected from previous experiments, none of the pupae in the next generation entered diapause even though they were reared in a strongly diapause-inducing environment of 12L:12D, 20°C. A group of larvae held in strongly diapause inducing conditions for the first four days after larviposition and then transferred to long day, 25°C until pupariation did not enter diapause (Fig. 4B). Progeny of females reared in this regime produced a high diapause incidence, indicating that four days of diapause-inducing conditions were inadequate to trigger the maternal effect. When females were raised in diapause-inducing conditions until pupariation, only a few pupae entered diapause (Fig. 4C). Progeny of flies that did not diapause show a highly reduced diapause level. Similar results were obtained when the flies received only ten days of strongly diapause-inducing conditions (Fig. 4D). Even at 25°C, exposure to short days resulted in progeny that were less likely to enter diapause (Fig. 4E). Clearly, larval exposure to short days can induce the maternal effect. Diapause itself is not a prerequisite.

4. Restoration of diapause among descendents

Crosses involving descendents of a female with a history of diapause consistently failed to yield diapause when the previous generation developed in a short-day regime (Fig. 5). When F₁ larvae of a female with a diapause history developed in a long day environment, the F₂ larvae were capable of entering diapause when exposed to the proper conditions. Thus, the capacity for diapause can be restored with one intervening generation at long day.
Figure 4. Pupal diapause incidence among progeny of parents exposed to various photoperiodic and temperature regimes. Except for A, all progeny were taken from females which do not enter diapause.
Females with a diapause history

Figure 5. Diapause incidence among descendents of a cross involving a female with a diapause history. SD designated a 12L:12D, 20°C (diapause inducing) environment during embryonic and larval stages. LD designates a 15L:9D, 25°C (nondiapause inducing) environment.
5. Exogenous hormone treatments

Neither juvenile hormone analog (JHA) nor ecdysone showed any clearcut effect upon diapause incidence regardless of the treated mother's history (Table 5). Nonetheless, the equivocal findings of these experiments and the narrow range of treatments indicate that further work is necessary.

All groups treated with ecdysone exhibited a considerable higher mortality rate than the control groups. This is most clearly noted in the females with no diapause history where mortality considerably increases in response to higher dosages. Ecdysone treatments also may have evoked a slight increased in diapause incidence among the progeny of females with a diapause history. This possible increase is detectable only because the control groups showed a 0% incidence.

Females with no diapause history, treated with acetone produced progeny that showed a sharply reduced level of diapause. This finding leads to uncertainty about the possible effects of JH as well as questions about what effect the acetone exerts. The tentative conclusion of these experiments is that the hormones exert, at best, only a slight effect although no statistical tests were applied to the data because of the problems encountered.

6. Comparison to S. crassipalpis

Preliminary investigations with S. crassipalpis indicate that a maternal effect may operate in this species as well, but the effect is not as pronounced. Females of this strain, reared as larvae in a long day environment, produced progeny with a very high diapause incidence
Table 5. The diapause incidence and mortality at 12L:12D, 20°C among progeny of female *S. bullata* treated with ecdysone or juvenile hormone.

<table>
<thead>
<tr>
<th>Diapause History</th>
<th>Treatment</th>
<th>No. of Females</th>
<th>No. of Larvae</th>
<th>Diapause Incidence (%)</th>
<th>Percent Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>No history</td>
<td>none</td>
<td>2</td>
<td>62</td>
<td>15.4</td>
<td>16.1</td>
</tr>
<tr>
<td></td>
<td>H₂O only</td>
<td>3</td>
<td>212</td>
<td>11.3</td>
<td>29.2</td>
</tr>
<tr>
<td></td>
<td>2 x 0.1 µg ecdysone</td>
<td>6</td>
<td>504</td>
<td>13.5</td>
<td>51.6</td>
</tr>
<tr>
<td></td>
<td>10 µg ecdysone</td>
<td>3</td>
<td>178</td>
<td>12.5</td>
<td>68.5</td>
</tr>
<tr>
<td></td>
<td>none</td>
<td>5</td>
<td>379</td>
<td>73.2</td>
<td>33.0</td>
</tr>
<tr>
<td></td>
<td>acetone only</td>
<td>6</td>
<td>431</td>
<td>21.0</td>
<td>20.4</td>
</tr>
<tr>
<td></td>
<td>2 x 0.5 µg JH</td>
<td>12</td>
<td>714</td>
<td>51.2</td>
<td>24.8</td>
</tr>
<tr>
<td></td>
<td>5.0 µg JH</td>
<td>5</td>
<td>400</td>
<td>24.1</td>
<td>34.5</td>
</tr>
<tr>
<td>History</td>
<td>none</td>
<td>6</td>
<td>237</td>
<td>0</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>2 x 0.1 µg ecdysone</td>
<td>7</td>
<td>314</td>
<td>2.6</td>
<td>26.1</td>
</tr>
<tr>
<td></td>
<td>10 µg ecdysone</td>
<td>6</td>
<td>325</td>
<td>2.3</td>
<td>19.4</td>
</tr>
<tr>
<td></td>
<td>none</td>
<td>4</td>
<td>168</td>
<td>0</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>acetone only</td>
<td>4</td>
<td>276</td>
<td>0</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>2 x 0.5 µg JH</td>
<td>2</td>
<td>120</td>
<td>0</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>5.0 µg JH</td>
<td>5</td>
<td>162</td>
<td>0.6</td>
<td>0.1</td>
</tr>
</tbody>
</table>
(98.1%, N = 256) at 12L:12D, 20°C. Under the same environmental conditions females with a diapause history produced progeny with a lower incidence of diapause (82.5%, N = 285). Similar results (Denlinger, unpublished) were obtained at 12L:12D, 25°C: starting with a diapause incidence of 39.1% (N = 325) in the parental generation, diapause incidence in the progeny of flies with a diapause history dropped to 25.6% (N = 258) and in the next generation dropped to 9.9% (N = 270).

D. Discussion

In response to short day exposure during their larval stage, females of Sarcophaga bullata prevent expression of diapause in their progeny. The transmittal of this maternal effect to the next generation occurs prior to ovulation and therefore directly affects the germ line. The capacity for diapause can be restored in a subsequent generation only by rearing an intervening generation under a long day photoperiod. The present experiments do not implicate either JH or ecdysone as agents for this effect, but further investigation will be required.

The virtual elimination of diapause among the progeny of females with a diapause history suggests that a critical component of the diapause mechanism is turned off or blocked. Genetic activity is extensive during oogenesis and maternally directed activity occurring at this time can affect the fate of post-fertilization events (Berry, 1982). The maternal effect might involve an alteration of a genetic regulatory event that occurs during oogenesis and ultimately affects the organism's capacity to diapause.
The nature of the maternal determinant operating in flesh flies has not yet been identified, but the regulatory mechanism for embryonic diapause in the silkmoth *Bombyx mori* may provide an insightful comparison. The photoperiod received by the female silkmoth during her embryonic and larval development determines whether her progeny will enter embryonic diapause (Fukuda, 1952; Hasegawa, 1952). In this species, a neurohormone from the female's subesophageal ganglion mediates the maternal effect (Yamashita et al., 1981). The situation in flesh flies is quite similar to *B. mori*: in both species a photoperiodic event occurring at an early developmental stage affects the incidence of diapause in the next generation. In several other species of Diptera (Cragg and Cole, 1952; Depner, 1962; Ring, 1967; Anderson, 1968; Vinogradova and Zinovjeva, 1972) and Hymenoptera (Schneiderman and Horowitz, 1958; Jackson, 1963; Ryan, 1965; Saunders, 1965) the maternal photoperiodic environment determines the incidence of diapause in the next generation, but in all of these cases the photoperiod acts directly on the adult female rather than on an earlier stage of development.

Attempts to implicate JH or ecdysone as agents of the maternal effect have failed. JH has been found in adult flies (Schooley et al., 1976) and ecdysone titers change during oogenesis (Briers and deLoof, 1980), suggesting that ecdysone plays a regulatory role at this time. Moreover, both hormones play an important regulatory role over other diapause processes (Zdarek and Denlinger, 1975; Gibbs, 1976; Walker and Denlinger, 1980; Denlinger, 1981a). The experiments performed here do not eliminate the possibility that either hormone acts in the adult to
alter diapause incidence among progeny, but the evidence from my studies gives no indication that they exert any effect on diapause.

The effects of acetone are enigmatic. A similar decrease in diapause incidence as a result of acetone treatments has been detected in *Nasonia vitripennis* (deLoof et al., 1979) and acetone acts to terminate diapause in *Sarcophaga* (Zdarek and Denlinger, 1975). A plausible explanation may be that this solvent activates a neurohormonal response that consequently alters these processes.

The maternal effect observed in *S. bullata* effectively prevents two successive generations of flies from entering diapause. The adaptive value of such a mechanism can only be appreciated in geographic areas that can support multivoltine populations of the fly. Flies emerging from diapause in the spring are thus assured of producing nondiapausing offspring. The timing of spring emergence is temperature dependent (Denlinger, 1972a; 1972b), and early in the spring flesh flies can be confronted with short daylengths that are diapause-inducing (Denlinger, 1972b). The maternal effect I now observe would prevent such an untimely entry into diapause. With the developmental consequences of springtime short daylength obliterated by the maternal effect, the species can safely invade a new temporal niche. Such a mechanism that could advance spring emergence may be of special importance to flesh flies. A long pupal diapause is achieved at a high cost: as the duration of pupal diapause increases, the ultimate reproductive output of the female declines (Denlinger, 1981c). Advancing spring emergence would shorten diapause and hence increase the number of fertile eggs produced by the female.
Chapter V
SUMMARY AND CONCLUSIONS

The present studies investigate environmental and genetic factors that affect the diapause characteristics. Flesh flies of the genus *Sarcophaga* enter pupal diapause in response to short days and thereby survive the climatic conditions associated with winter. At other times entry into diapause is nonadaptive. Therefore, the mode of inheritance plays an essential role in maintaining the ability to enter and avoid diapause at the appropriate times.

The importance of genetic factors is established by the large differences in response obtainable through selection from a single original strain. Because of the large genetic variability that presumably exists in natural populations, diapause attributes may vary greatly as a consequence of selective forces in given locales. However, phenotypic differences arise from gene differences at many loci which interact with each other and with the environment. These interactions reflect the complexity of the diapause phenotype.

These genetic factors appear to affect other aspects of development besides the capability to enter diapause. Selection for differences in developmental rate also alters diapause capability and diapause duration. Whether these are pleiotropic effects or the consequence of linkage is
not known. Nonetheless, these traits may be adaptively linked and serve to maximize the fitness of a population by insuring that a longer diapause stage accompanies a high capability to enter diapause. This functional association would be especially adaptive in northern localities where early entrance and late emergence increases the chances for survival through winter.

The elimination of diapause among progeny of females exposed as larvae to short days points out the importance of environmental factors in pre-adult stages. These can act to regulate the response to short days in such a way that diapause is avoided in the spring and entered only in the autumn. Developmentally, the maternal effect apparently alters genetic expression during oogenesis, and thereby alters later responsiveness to photoperiod.

Future studies with natural populations of S. bullata can verify the importance of these hereditary aspects of diapause and enhance our understanding of diapause as an adaptive mechanism. Furthermore, diapause is a developmental phenomenon and the physiologic events associated with diapause have been extensively investigated. Future work can pursue the relationship between genetic activity and the neuroendocrine events associated with this developmental event in S. bullata.
APPENDIX A

Raw Data for Chapter II

The following data were collected from individual females. The number of diapausing pupae appear in the numerator, the total number of offspring in the denominator. The number of dead pupae are in parentheses alongside the data for each female. These results were used for Table 1 and Figure 1. H denotes line selected for high diapause incidence and L denotes line selected for low incidence:

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>H x H</td>
<td>18/25, 53/60, 28/28, 33/34, 43/43, 17/17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L x L</td>
<td>16/42 (1), 26/55 (1), 1/45 (1), 4/57 (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H x L</td>
<td>36/65, 7/27, 20/57</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L x H</td>
<td>6/13 (2), 18/37 (1), 2/8 (0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H x L - F₁</td>
<td>18/46 (0), 5/41 (2), 32/70 (1), 9/54 (3), 14/69 (26), 12/36 (1), 29/59 (2), 5/43 (1), 14/37 (49)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L x H - F₁</td>
<td>59/98 (1), 5/17 (2), 21/43 (5), 23/72 (6), 48/83 (2), 31/68 (5), 19/56 (0), 42/95 (7), 54/98</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(H x L) x H</td>
<td>3/8 (0), 28/44 (0), 16/34 (2), 26/29 (1), 25/35 (12)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H x (H x L)</td>
<td>86/88 (2), 35/68 (2), 10/12 (1), 56/63 (2), 28/52 (6), 75/83 (15), 75/79 (5), 48/65 (5), 34/48 (0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(L x H) x H</td>
<td>67/84 (0), 37/37 (7), 39/78 (22), 38/64 (40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H x (L x H)</td>
<td>41/45 (1), 9/9 (5), 61/61 (28), 37/66 (5), 28/41 (36), 26/37 (7), 47/94 (6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(H x L) x L</td>
<td>15/54 (1), 20/42 (7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L x (H x L)</td>
<td>70/81 (12), 20/49 (1), 14/35 (9), 27/50 (3), 6/46 (37), 48/89 (5), 11/122 (3), 10/58 (4), 0/27 (1), 1/78 (5), 23/54 (0)</td>
<td></td>
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</table>
APPENDIX A. Continued

<table>
<thead>
<tr>
<th>Structure</th>
<th>Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>((L \times H) \times L)</td>
<td>20/104 (1), 4/48 (0), 14/77 (0), 20/93 (16), 20/71 (5), 21/65 (5)</td>
</tr>
<tr>
<td>(L \times (L \times H))</td>
<td>17/66 (8), 20/53 (7), 12/55 (7), 9/34 (8), 4/42 (0)</td>
</tr>
</tbody>
</table>
APPENDIX B
Raw Data for Chapter III

The data used for calculating the mean duration of the larval stage at 15L:9D, 25°C (Table 2 and Figure 2) for the strain selected for late pupariation and the unselected strain are given below:

<table>
<thead>
<tr>
<th>Days after Larviposition</th>
<th>Number of Puparia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Selected Strain</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>142</td>
</tr>
<tr>
<td>7</td>
<td>64</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>232</td>
</tr>
</tbody>
</table>

The data for mean duration of the larval stage at 12L:12D, 20°C are given below (Table 2 and Figure 2):

<table>
<thead>
<tr>
<th>Days after Larviposition</th>
<th>Number of Puparia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Selected Strain</td>
</tr>
<tr>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>21</td>
<td>31</td>
</tr>
<tr>
<td>22</td>
<td>32</td>
</tr>
<tr>
<td>23</td>
<td>31</td>
</tr>
<tr>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>26</td>
<td>15</td>
</tr>
<tr>
<td>27</td>
<td>9</td>
</tr>
<tr>
<td>28</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>3 - did not pupate</td>
</tr>
<tr>
<td></td>
<td>216</td>
</tr>
</tbody>
</table>

59
The following data were used to calculate the diapause incidence in the two strains. Numerator indicates the number of diapausing pupae, the denominator indicates total pupae counted from individual females (Table 2):

<table>
<thead>
<tr>
<th>Selection</th>
<th>53/60, 28/28, 33/34, 43/43, 17/17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unselected</td>
<td>29/54, 18/41, 19/30, 63/77</td>
</tr>
</tbody>
</table>

To calculate the mean time from transfer to 25°C (on day 60 after larviposition) until differentiation of antennal discs (the termination of diapause), the following data were used (Table 2 and Figure 3):

<table>
<thead>
<tr>
<th>Days after Larviposition</th>
<th>Developing Pupae</th>
<th>Days after Larviposition</th>
<th>Developing Pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Selected</td>
<td>Unselected</td>
<td>Selected</td>
</tr>
<tr>
<td>65</td>
<td>0</td>
<td>5</td>
<td>91</td>
</tr>
<tr>
<td>66</td>
<td>0</td>
<td>5</td>
<td>92</td>
</tr>
<tr>
<td>67</td>
<td>0</td>
<td>21</td>
<td>93</td>
</tr>
<tr>
<td>68</td>
<td>1</td>
<td>16</td>
<td>94</td>
</tr>
<tr>
<td>69</td>
<td>1</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>70</td>
<td>0</td>
<td>7</td>
<td>96</td>
</tr>
<tr>
<td>71</td>
<td>0</td>
<td>4</td>
<td>97</td>
</tr>
<tr>
<td>72</td>
<td>0</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>73</td>
<td>0</td>
<td>3</td>
<td>99</td>
</tr>
<tr>
<td>74</td>
<td>0</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>75</td>
<td>2</td>
<td>0</td>
<td>101</td>
</tr>
<tr>
<td>76</td>
<td>0</td>
<td>2</td>
<td>102</td>
</tr>
<tr>
<td>77</td>
<td>0</td>
<td>3</td>
<td>103</td>
</tr>
<tr>
<td>78</td>
<td>0</td>
<td>4</td>
<td>104</td>
</tr>
<tr>
<td>79</td>
<td>0</td>
<td>0</td>
<td>105</td>
</tr>
<tr>
<td>80</td>
<td>0</td>
<td>1</td>
<td>106</td>
</tr>
<tr>
<td>81</td>
<td>3</td>
<td>0</td>
<td>107</td>
</tr>
<tr>
<td>82</td>
<td>0</td>
<td>0</td>
<td>108</td>
</tr>
<tr>
<td>83</td>
<td>0</td>
<td>2</td>
<td>109</td>
</tr>
<tr>
<td>84</td>
<td>0</td>
<td>0</td>
<td>110</td>
</tr>
<tr>
<td>85</td>
<td>2</td>
<td>0</td>
<td>111</td>
</tr>
<tr>
<td>86</td>
<td>1</td>
<td>0</td>
<td>112</td>
</tr>
<tr>
<td>87</td>
<td>1</td>
<td>2</td>
<td>113</td>
</tr>
<tr>
<td>88</td>
<td>0</td>
<td>0</td>
<td>114</td>
</tr>
<tr>
<td>89</td>
<td>2</td>
<td>0</td>
<td>115</td>
</tr>
<tr>
<td>90</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

The selection experiment was initiated with larvae collected on January 2, 1981. The sixth generation selected for late pupariation produced larvae on August 4, 1981. On August 7, the seventh generation in the unselected group produced larvae. These dates were used to calculate the mean generation length (Table 2).
APPENDIX C
Raw Data for Chapter IV

The following data were used to calculate the diapause incidence for the crosses given in Table 3. For all data here, the numerator indicates the number of diapausing pupae. The denominator indicates the total number of pupae for each female: The number in parentheses indicates the number of dead pupae:

N x N
  a. 76/80, 26/85, 20/31, 51/53, 14/16
  b. 9/18 (16), 7/16 (10), 8/18 (39), 16/26 (13), 10/36 (6), 18/34 (9)

D x D
  a. 0/43, 0/52, 0/53
  b. 0/70, 0/40

N x D
  a. 73/75, 40/49, 47/60, 27/47
  b. 18/31, 29/38, 16/30 (1), 26/84 (2), 29/34 (0)

D x N
  a. 0/61, 0/60, 0/66, 0/44
  b. 0/10 (2), 0/50 (7), 0/26 (0), 0/46 (1), 0/52 (3), 0/35 (5)

The data for Table 4 are given below:

Days After Ovulation

Females with a Diapause History

<table>
<thead>
<tr>
<th>Days</th>
<th>0/1, 0/9</th>
<th>0/3, 0/34, 0/19, 0/3</th>
<th>1/7, 0/19, 0/45, 0/15, 0/15, 0/20, 0/5, 0/37, 0/22, 0/22, 0/53, 0/11, 0/41, 0/23, 0/33, 0/14</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0/1, 0/9</td>
<td>0/3, 0/34, 0/19, 0/3</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0/3, 0/34, 0/19, 0/3</td>
<td>1/7, 0/19, 0/45, 0/15, 0/15, 0/20, 0/5, 0/37, 0/22, 0/22, 0/53, 0/11, 0/41, 0/23, 0/33, 0/14</td>
<td></td>
</tr>
</tbody>
</table>

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Appendix C. Continued

Females with a Diapause History

<table>
<thead>
<tr>
<th></th>
<th>0/4, 0/16, 0/26, 0/36, 0/20, 0/28, 0/24, 0/13</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0/19, 0/7</td>
</tr>
<tr>
<td>4</td>
<td>0/40, 0/48, 1/73, 0/33, 3/82, 0/75, 0/61, 2/91, 0/39, 0/92, 0/51, 0/73, 0/59, 0/7, 0/43, 0/18</td>
</tr>
</tbody>
</table>

Females with No Diapause History

<table>
<thead>
<tr>
<th></th>
<th>19/20, 0/2, 6/7</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7/9, 0/4, 7/19, 3/15, 11/12, 2/4, 12/17, 29/46</td>
</tr>
<tr>
<td>1</td>
<td>37/51, 1/9, 2/21</td>
</tr>
<tr>
<td>2</td>
<td>17/38, 9/26, 27/27</td>
</tr>
<tr>
<td>3</td>
<td>21/43, 11/33, 17/19</td>
</tr>
<tr>
<td>4</td>
<td>39/66, 48/95, 13/19, 30/92, 26/82, 78/82, 45/47, 47/60, 73/75, 40/49, 72/73, 27/40, 80/94, 68/90, 12/70, 2/90, 1/82, 22/63, 2/82, 0/59, 42/51, 18/70, 73/91, 98/102, 83/86, 92/109, 84/89, 61/61, 45/48, 52/60.</td>
</tr>
</tbody>
</table>

The data used for Figure 4 are given below:

<table>
<thead>
<tr>
<th>Diapause Incidence</th>
<th>Progeny Diapause Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. 12/99, 27/58, 43/93, 59/73, 39/95</td>
<td>0/15, 0/81, 0/42, 0/58, 0/16</td>
</tr>
<tr>
<td>B. 0/77</td>
<td>15/41, 12/30, 22/45, 4/26</td>
</tr>
<tr>
<td>C. 3/26</td>
<td>0/27, 0/89, 0/15, 0/61, 14/49</td>
</tr>
<tr>
<td>D. 10/83</td>
<td>0/46, 3/24, 0/44, 4/32, 1/70, 0/25, 0/25, 0/76, 2/33, 22/71, 1/62</td>
</tr>
<tr>
<td>E. 0/81, 0/74</td>
<td>0/70, 3/82, 24/88, 4/34, 22/96, 0/15</td>
</tr>
</tbody>
</table>
For Figure 5, the following data were recorded:

\[ F_1^{(SD)} \quad 0/53, 0/57, 0/27 \]
\[ F_2^{(SD)} \quad 0/87, 0/89, 0/87, 0/28 \]
\[ F_3^{(SD)} \quad 0/67, 0/56 \]
\[ F_4^{(SD)} \quad 0/110, 0/15, 0/80, 0/12, 0/98, 0/97 \]
\[ F_1^{(LD)} \quad \text{No data recorded} \]
\[ F_2^{(LD)} \quad 58/60 (10), 19/96 (14), 0/26 (9), 11/15 (5), 38/87 (10), 10/16 (15), 36/50 (1), 27/61 (1), 30/30 (3) \]

For Table 5:

Females with a Diapause History

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>0/10 (2), 0/50 (7), 0/26 (0), 0/46 (1), 0/52 (3), 0/35 (5)</td>
</tr>
<tr>
<td>H2O only</td>
<td>not applied</td>
</tr>
<tr>
<td>2 x 0.1 μg ecdysone</td>
<td>0/21 (11), 1/51 (4), 1/1 (0), 1/11 (0), 0/72 (27), 3/39 (1), 0/37 (39)</td>
</tr>
<tr>
<td>10 μg ecdysone</td>
<td>0/79 (2), 0/11 (2), 4/64 (0), 0/30 (28), 2/51 (16), 0/29 (15)</td>
</tr>
<tr>
<td>No treatment</td>
<td>0/26 (0), 0/46 (1), 0/52 (3), 0/35 (5)</td>
</tr>
<tr>
<td>Acetone only</td>
<td>0/94 (2), 0/84 (6), 0/72 (12), 0/6</td>
</tr>
<tr>
<td>2 x 0.5 μg JH</td>
<td>0/21, 0/90 (9)</td>
</tr>
<tr>
<td>5.0 μg JH</td>
<td>1/53 (0), 0/19 (0), 0/22 (0), 0/23 (1), 0/43 (1)</td>
</tr>
</tbody>
</table>
Appendix C. Continued.

<table>
<thead>
<tr>
<th>Females with No Diapause History</th>
<th>8/52 (10)</th>
<th>9/42 (52), 0/9 (4), 8/82 (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>8/52 (10)</td>
<td>9/42 (52), 0/9 (4), 8/82 (6)</td>
</tr>
<tr>
<td>H₂O only</td>
<td>4/7 (19), 3/16 (59), 0/33 (44)</td>
<td>72/97 (20), 20/36 (16), 17/29 (12), 45/52 (9), 32/40 (68)</td>
</tr>
<tr>
<td>2 x 0.1 µg ecdysone</td>
<td>0/4 (104), 23/94 (20), 4/58 (37), 5/8 (17), 0/6 (67), 1/74 (15)</td>
<td>15/80 (6), 10/17 (1), 18/61 (8), 10/46 (44), 18/71 (21), 1/68 (8)</td>
</tr>
<tr>
<td>10 µg ecdysone</td>
<td>4/7 (19), 3/16 (59), 0/33 (44)</td>
<td>72/97 (20), 20/36 (16), 17/29 (12), 45/52 (9), 32/40 (68)</td>
</tr>
<tr>
<td>No treatment</td>
<td>4/7 (19), 3/16 (59), 0/33 (44)</td>
<td>72/97 (20), 20/36 (16), 17/29 (12), 45/52 (9), 32/40 (68)</td>
</tr>
<tr>
<td>Acetone only</td>
<td>29/58 (13), 32/64 (19), 1/23 (68), 1/1 (0), 39/74 (14), 31/92 (13), 20/32 (4), 7/32 (12), 15/16 (1), 10/42 (32), 51/56 (8), 68/105 (6)</td>
<td>15/80 (6), 10/17 (1), 18/61 (8), 10/46 (44), 18/71 (21), 1/68 (8)</td>
</tr>
<tr>
<td>2 x 0.5 µg JH</td>
<td>19/80 (6), 10/17 (1), 18/61 (8), 10/46 (44), 18/71 (21), 1/68 (8)</td>
<td>29/58 (13), 32/64 (19), 1/23 (68), 1/1 (0), 39/74 (14), 31/92 (13), 20/32 (4), 7/32 (12), 15/16 (1), 10/42 (32), 51/56 (8), 68/105 (6)</td>
</tr>
<tr>
<td>5.0 µg JH</td>
<td>27/58 (13), 4/48 (22), 4/27 (44), 9/45 (36), 19/84 (23)</td>
<td>29/58 (13), 32/64 (19), 1/23 (68), 1/1 (0), 39/74 (14), 31/92 (13), 20/32 (4), 7/32 (12), 15/16 (1), 10/42 (32), 51/56 (8), 68/105 (6)</td>
</tr>
</tbody>
</table>
A Procedure for Isolating Diapause Mutants

An unsuccessful attempt was made to generate mutants with ethyl methane sulfonate (EMS) that would show a decrease in diapause capability in a strongly diapause inducing environment (12L:12D, 20°C). The failure of this attempt was partially the consequence of the maternal effect (Chapter IV).

The following procedure would be recommended for isolation of low diapause and nondiapause mutants in S. bullata. All EMS procedures follow the methods described by Lewis and Bacher (1968) except that EMS would be applied to sugar or sweetened water that flies feed upon. The flies utilized should show an incidence of diapause that is consistently high. The lines should be inbred to increase initial homozygosity and decrease variability in diapause incidence:

Procedures:

(1) Treat males with EMS and mate these to a few (no more than 5) females reared in long-day (15L:9D, 25°C) conditions.

(2) Take the progeny of individual crosses, rear to adulthood and produce sibling matings.

(3) Again, take progeny of individual crosses and rear larvae in long day conditions.

(4) Cross siblings at 12L:12D, 25°C, take larvae from individual crosses and raise offspring at 12L:12D, 20°C. Any groups that show a low diapause response are mutant candidates.

Selection for mutants that increase diapause incidence can be performed similarly utilizing a strain showing an inherently low diapause incidence or that shows a low incidence as a consequence of the maternal effect (Chapter IV).

Note that one female can produce 40-50 crosses in the first generation and 40-50 per female can arise from these crosses. The procedure requires a large amount of rearing resources.


