Genetic differences in pupal diapause incidence between two selected strains of the flesh fly

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Abstract:

This study assesses the importance of genetic differences that underlie differences in pupal diapause incidence among two lines of the flesh fly, *Sarcophaga bullata*. Lines of high and low diapause Incidence were derived through selection and subsequent Inbreeding from an original strain and the diapause incidence was observed among F_1, F_2, and backcross progeny in a strongly diapause-inducing environment. Results consistently revealed patterns indicating that diapause capability is greatly dependent on heritable factors, although the patterns of Inheritance are not additive. The observed differences in diapause levels resulting from artificial selection imply that local natural populations exhibit a large amount of variability in response to diapause-inducing environmental factors. A relatively small number of gene loci are likely to control the diapause response in this species.

Article:

Many insects enter a developmental arrest (diapause) that allows them to survive harsh seasonal conditions. Genetic studies derived from a variety of species indicate that diapause characteristics respond rapidly to artificial selection[11], show high levels of heritability as revealed by interstrain crosses[3,12,15], and can be altered by single gene locus mulations[18]. Strains of diverse natural origin show differences in diapause traits that presumably arise from genotypic differences shaped by selective forces[1,13].

Although studies utilizing natural strains obviously have merit, genetic analyses using such strains suffer because the variability of interstrain crosses cannot be controlled, Diapause traits of these strains show varying degrees of polymorphism that may reflect underlying genotypic differences[2]. The observed differences may be complicated by genetic and environmental interactions at loci that influence diapause, and consequently genetic differences may prove difficult to evaluate. Two-way artificial selection and subsequent inbreeding serve to maximize genetic differences between two lines and increase the likelihood of homozygosity at loci that affect diapause. By crossing inbred strains for high and low diapause that have been selected from the same original strain, genetic differences giving rise to the different levels of response can be more precisely defined. Using such an approach with the spider mite *Tetranychus urticae*, Helle[8] was able to correlate differences in photoperiodic response with major gene differences.

In this study with the flesh fly, *Sarcophaga bullata*, we utilize a similar approach. *S. bullata* enters pupal diapause in response to short daylength and cool temperatures experienced during embryogenesis and early larval life[4,5]. We have developed lines for high and low diapause incidence and utilized these lines to evaluate gene differences in the diapause response by interstrain crosses.

Materials and Methods

Selected lines were derived from a strain of *S. bullata* that originated in Lexington, Massachusetts in 1974. Prior to selection the strain yielded a diapause incidence 47.0 percent when reared at 12L:12D (light:dark cycle), 23°C, Procedures for rearing flies were previously described[4].

To develop a line with low diapause incidence (L), adults of the original strain were mass mate4 and maintained at 12L:12D, 25°C, females were allowed to larviposit on the 11th day after eclosion, and larvae were reared at 12L: 12D, 23°C. Approximately 30 days after larviposition, pupae were examined for diapause using the criteria described by Fraenkel and Hsiao[7]. Twenty individuals that had not entered diapause were randomly selected and mated after adult eclosion. Progeny were reared in the same way and the selection procedure was repeated for three more generations. By the fifth generation, the diapause incidence in this regime was 0 percent. Therefore, from the fifth through the twelfth generation, larvae were reared and selected at 12L; 12D, 20°C, an environment that induces a higher diapause incidence and produced a low level of diapause in the L line, To increase the likelihood of homozygosity, larvae reared in the 11th, 12th, and 13th generations originated from a single female. In this species, rearing the mother in short daylength during her larval stage reduces pupal diapause in her progeny[9]. To eliminate the possibility of this environmentally induced reduction of diapause (the maternal effect), larvae of the 13th generation were reared at 15L:9D, 25°C, Consequently, no larvae entered diapause and no selection for diapause traits was possible. The adult siblings that comprised the 13th generation were utilized as the low diapause line for interline crosses and were transferred to an environment of 12L: 12D, 25°C within a day after eclosion.

The maternal effect prevents expression of diapause in two successive generations. Thus, a line with high-diapause incidence could not be selected directly. But, since larval developmental rate correlates with pupal diapause capability[10], a high diapause line (H) was selected indirectly over six generations by mating individuals that had pupariated relatively late in a nondiapause-inducing environment.

Interline crosses (H X L and L X H, female designated on left) were made by mass mating males of one line and females of the other at 12L.:12D, 25°C. Larvae were collected from individual females and maintained at 12L: 12D, 20°C. To avoid the environmentally induced reduction in diapause associated with the maternal effect, hybrids for F_1 crosses and backcrosses, as well as members of the H and L lines, were mated in long-day conditions. The progeny of these crosses were mated in 12L; 12D, 25°C to produce data for the following groups: H × (H × L), H × L × H), (H × L) × H,(L × H) × H, L × (H × L), L × (L × H), (H × L) × L, (L × H) × L, (H × L) × (H × L), and (L × H) × (L × H). Progeny from these crosses were placed in 12L:12D, 20°C after larviposition and later scored for diapause.

Results

Diapause incidence in progeny from all the crosses of lines selected for high (H) and low (L) diapause is shown in Table I. Although differences exist within the F_1, F_2, and backcross groups, data were consolidated into four groups for analysis: F_1 progeny, F_2 progeny, backcrosses to the high line, and backcrosses to the low line, Since percentages in the cumulative groups ranged between 28.5 and 73.6 percent an arcsin transformation was not used for statistical analyses[16].

The simplest genetic model for testing these results assumes additive inheritance with no dominance. With no dominance the F_1 progeny should show a diapause incidence inter-mediate between the two parental lines (58.6 percent). The actual pooled F_1 incidence (42.9 percent) varies significantly from the results expected with no dominance ($P(z) < 0.01$). We thus tested the hypothesis that the inheritance of diapause among these lines is additive and the low trait is incompletely dominant.

The probability (d) that a larva will enter diapause when endowed with a particular genotype is binomially distributed. Therefore, the mean incidence for a sample is $\Sigma_{i=1}^{N} d/N$ and the variance is $d(1 - d)/N$ 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 was generated with the following equations (D indicates observed diapause incidence of the designated population):

$$E_{F_2} = D_H/4 + D_{F_1}/2 + D_L/4$$

$$E_{BCH} = \frac{D_H + D_{F_1}}{2}$$

$$E_{BCL} = \frac{D_L + D_{F_1}}{2}.$$

Table I. Pupal diapause incidence at 12L:12D, 20°C of parental, F_1, and backcrosses using members of line of *S. bullata* selected for high diapause incidence (H) and low diapause incidence (L)

Cross (♀ × ♂)	No. females	No. larvae	Diapause incidence (%)	Standard deviation (%)	Expected value (additive model)	Deviation (δ)	Variance of δ (V_δ)	Test statistic
H × H	6	207	92.7	3.2				
L × L	5	220	24.5	8.4				
H × L	3	149	42.2					
L × H	3	58	44.8					
F_1 (Cum.)	6	207	42.9	11.8				
(H × L) × (H × L)	9	409	33.7					
(L × H) × (L × H)	9	630	47.9					
F_2 (Cum.)	18	1039	42.3	2.3	50.8	0.3	9.6×10^{-3}	3.45**
H × (H × L)	9	558	80.1					
H × (L × H)	7	353	70.5					
(H × L) × H	5	150	65.3					
(L × H) × H	4	263	68.8					
BCH (Cum.)	25	1324	73.6	1.4	67.8	0.1	2.1×10^{-3}	2.52*
L × (H × L)	11	689	33.3					
L × (L × H)	5	250	24.8					
(H × L) × L	2	96	36.4					
(L × H) × L	6	458	21.6					
BCL (Cum.)	24	1493	28.5	1.3	33.7	0.1	5.3×10^{-3}	1.43 n.s.

* $P(Z) < 0.05$ ** $P(Z) < 0.01$

If the observed and expected values in the F_2 group arc equal, then:

$$E_{F_2} = D_{F_2}$$

and:

$$D_{F_2} - D_H/4 - D_{F_1}/2 - D_L/4 = 0.$$

By algebraic manipulation,

$$4D_{F_2} - D_H - 2D_{F_1} - D_L = 0.$$

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

$$4D_{F_2} - D_H - 2D_{F_1} - D_L = \delta_{F_2}$$

$$2D_{BCH} - D_H - D_{F_1} = \delta_{BCH}$$

$$2D_{BCL} - D_L - D_{F_1} = \delta_{BCL}.$$

The variance of the deviation, V_δ, also can be calculated:

$$V\delta_{F_2} = 16V_{F_2} + V_H + 4V_{F_1} + V_L$$

$$V\delta_{BCH} = 4V_{BCH} + V_H + V_{F_1}$$

$$V\delta_{BCL} = 4V_{BCL} + V_L + V_{F_1}$$

The resulting test statistic, $\delta/\sqrt{V_\delta}$, is the standard normal variate. The value of this statistic shows that the BCL incidence fits an additive model. By contrast, the F_2 ($P(Z) < 0.01$) and BCH ($P(Z) < 0.05$) do not fit an additive model, indicating that genetic and genetic-environmental interactions are involved. The actual data and the data predicted by an additive model with incomplete dominance of the low diapause trait are shown in Figure 1. As indicated by the diapause incidences detected in the progeny and backcrosses, genetic factors clearly play an important role in determining the capability for diapause.

Discussion

Results from interline crosses generally do not fit an additive hypothesis and suggest that a number of genetic

factors are associated with differences in diapause capability. The diapause incidences of progeny consistently fall between the levels observed in the parents and the consistency of these results clearly implies that genetic factors largely determine the di-a pause capability in a given environment.

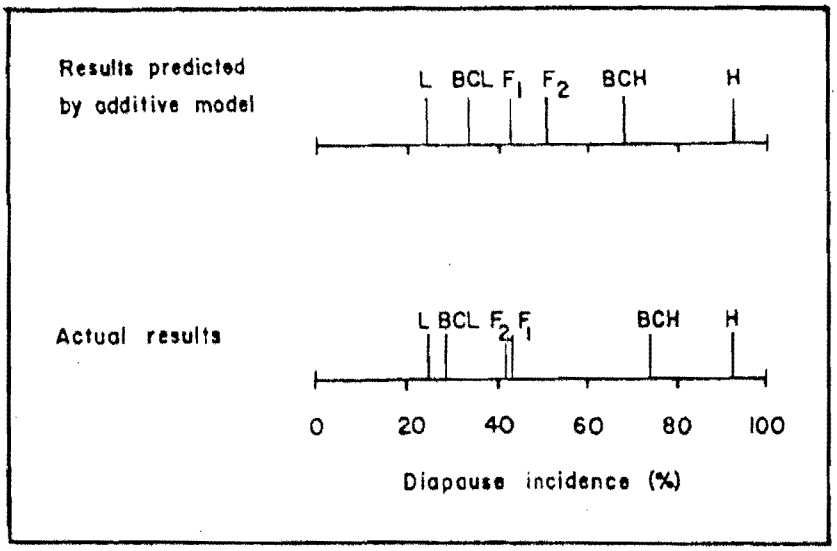

FIGURE 1 A comparison of observed diapause incidence at 12L:12D, 20°C among progeny of crosses involving lines of *Sarcophaga bullata* selected for high (H) and low (L) diapause incidence with results predicted by an additive model with incomplete dominance. Actual data for H, L, and F_1 were used to calculate expected values for F_2 and the backcrosses to the high (BCH) and low (BCL) lines.

The genetic variability detected between the two investigated lines does not reflect the total possible genetic variance in diapause capability, Many loci that affect diapause capability may contain no functional allelic differences in the original strain and therefore would remain undetected by interstrain analysis. Inbreeding, inadvertent selection, and the small founder population would tend to reduce the genetic variability and therefore, the detectable phenotypic variability.

A substantial proportion of the detectable variance for diapause capability depends on genetic-environmental interactions. In our study, crosses were performed in only one environmental regime, a regime that was chosen primarily because it permitted analysis of large differences in diapause incidence. These interactions obviously affect detectable genetic differences; in a nondiapausing environment no measurable differences in capability can be found. In other environments, however, it is possible that genotypic differences not detectable in the present study might be expressed and conversely, other genetic differences suppressed. The patterns of inheritance for diapause capability may thus appear different in another environment. This possibility may be important ecologically, especially since photoperiod and temperature, the two environmental factors known to influence diapause to the greatest extent, vary cyclically in natural situations. The importance of gene-environment interactions can be evaluated best by analyzing crosses over a range of environments between selected lines derived from a number of naturally isolated strains. Although such an evaluation would be extensive, it would pro-vide a better indication of the degree of polymorphism that exists in natural populations for diapause traits as well as provide an indication of the importance of genetic factors over a range of environmental conditions. This approach has been utilized to analyze the evolutionary aspects of seasonal phenology in a number of species[6,13,17].

On the organismal level, as on the populational level, diapause in *S. bullata* is complex and is associated with several developmental and physiological events. The selected lines were not evaluated for differences in sensitivity to photoperiod or temperature. It is possible, as suggested by Hoy[11], that strains selected for changes in response to temperature would differ genotypically from those selected for changes in photoperiodic response. Such a result would indicate that different subsets of genetic factors affect these components of the total response. But, evidence gathered from studies of the 11 line indicated a strong correlation between larval developmental rate, diapause incidence, and diapause duration, suggesting that these traits arc affected by

common genetic elements[10], It appears likely that the metabolic, hormonal, and developmental events that comprise the diapause "syndrome" in *S. bullata* depend on the activity of a relatively small number of regulatory, interacting loci or a single-gene locus, similar to the one that regulates photoperiodic response in *Drosophila littoralis*[14]. Isolation and characterization of mutants in this species could provide a useful mechanism to probe genetic activity underlying the diapause response.

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