Mendelian Inheritance of Pupal Diapause in the Flesh Fly, Sarcophaga bullata

BING HAN AND DAVID L. DENLINGER

From the Department of Entomology, The Ohio State University, 318 West 12th Avenue, Columbus, OH 43210.

Address correspondence to David L. Denlinger at the address above, or e-mail: denlinger.1@osu.edu.

Pupal diapause (dormancy) in the flesh fly, Sarcophaga bullata, is induced by short-day photoperiods and low temperature. In this study, the inheritance mode of diapause was investigated by crossing a nondiapausing (nd) strain of S. bullata with 2 diapausing strains having different diapause capacities. The results consistently indicated that diapause incidence is inherited in a simple Mendelian pattern, thus a single gene or a small gene cluster linked to the photoperiodic clock controls the seasonal response of diapause. The fact that the nd strain lacked daily rhythmicity in adult eclosion and showed altered expression of 2 circadian clock genes suggests that the photoperiodic and circadian clocks are related through a shared molecular component in S. bullata.

Key words: circadian clock, diapause, Mendelian inheritance, photoperiodic response

Diapause is a form of dormancy in insects, characterized by a suite of physiological changes that confer survival advantages during unfavorable seasonal conditions, such as the low temperatures of winter (Tauber et al. 1986; Denlinger 2002). The occurrence of diapause is determined by various environmental inputs, but most insects residing in temperate regions use photoperiod as the primary cue for monitoring seasonal changes (Saunders 2002). Thus, a hypothetical photoperiodic clock system is proposed to be responsible for the seasonal timing of diapause (Saunders and Sutton 1969).

It has been known for quite some time that diapause has a genetic basis (e.g., Waldbauer and Sternberg 1973; Hayes et al. 1987; Roff and Bradford 2000). The fact that much geographic and latitudinal variation in photoperiodism has been observed suggests that there is a great amount of genetic polymorphism for this trait within populations. Various adaptive clines are formed under different local selection pressure (Riihimaa et al. 1996).

Although the heritable characteristic of diapause has been confirmed, conclusions obtained from different species about the mode of inheritance are somewhat contradictory. Fortunately, the availability of different phenotypes provides the possibility of studying the genetic mode by reciprocal crossing. Based on the fact that diapause is characterized by diverse inherent traits in different species, is induced and regulated by various seasonal environmental changes, and is coordinated by distinct endocrinological factors in different insect species (Danks 1987; Yamashita 1996; Lee et al. 1997), it has been suggested that a polygenic system is involved in diapause determination (Wipking and Kurtz 2000), a view that is partially supported by results obtained in the rice stem maggot, Chilo oryzae (Takeda 1998). Diapause-related genes are sex linked in some species, such as the European corn borer, Ostrinia nubilalis (Reed et al. 1981). Inheritance can be biased toward either parent depending on the species. For instance, the diapause incidence is mainly determined maternally in the blowfly, Calliphora vicina (McWatters and Saunders 1996, 1997).

More evidence, however, suggests that diapause can be determined by only a small subset of diapause-related genes. Several experiments show that inheritance of diapause does not fit an additive model and that the capacity for diapause is genetically transmitted in a manner of incomplete dominance (Henrich and Denlinger 1983; Kim et al. 1995). In several cases, the number of major “diapause-controlling” genes may be as few as one, as suggested by the simple Mendelian inheritance pattern exhibited by progeny crosses. For example, in crosses of the spider mite, Tetranychus pueraridola (Suwa and Gotoh 2006) and the linden bug, Pyrrhocoris apterus (Dolezel et al. 2005), the segregation rate in hybrids was close to 3:1, implying a major role for one gene or a small gene cluster in diapause induction. Incomplete or complete dominant effects may also be caused by gene integration, based on the fact that multiple diapause-related genes tend to be closely linked, as shown in mosquitoes of the Culex pipiens complex (Mori et al. 2007).

The flesh fly, Sarcophaga bullata, overwinters in pupal diapause, a state induced by short daylength and low temperature (Denlinger 1972; Gnagey and Denlinger 1984). Strains of this fly showing different diapause responses are available. In the present study, we crossed a nondiapausing (nd) strain with 2 different diapausing strains. The diapause responses of the hybrids strongly support a mode of Mendelian inheritance of diapause, suggesting a single gene...
or a cluster of tightly linked genes that are responsible for photoperiodic induction of diapause.

Materials and Methods

Insects

Three colonies of *S. bullata* Parker were maintained as described (Denlinger 1972). In addition to an nd strain previously described (Goto et al. 2006), a novel strain exhibiting low diapause (ld) incidence was also reared; this strain was derived from a colony of wild-type (wt) flies described by Goto et al. (2006). In addition, a wt colony with a high diapause incidence recently collected in Alabama (30°/C176N latitude) was used. Stocks of all these colonies were reared throughout development in long-day photoperiod conditions (light:dark 15:9 h) at 25°/C176C.

Reciprocal Crossing

Reciprocal crosses between the nd strain and each of the 2 diapausing strains were performed. To start the crossing, the sexes were separated on the first day of adult eclosion, after which 20 males flies of one strain and 20 female flies of the other strain were combined for each cross. Hybrids of the first (F1) and second generation resulting from self-crossing of F1 flies (F2) as well as progeny resulting from backcrossing with the nd strain (BcN) and the diapausing strain (BcD) were obtained from these crosses.

Diapause Incidences

To provide diapause-inducing conditions and to eliminate a possible maternal effect (Denlinger 1971; Henrich and Denlinger 1982), flies of each individual cross were transferred at adult eclosion from a long-day photoperiod to light:dark 12:12 h at 25 °C for adult life and light:dark 12:12 h at a low temperature for the larvae and pupae that the adults produced. Because temperatures that are too low are lethal and temperatures that are too high reduce the diapause incidence, the diapause-inducing low temperature was carefully chosen with pilot experiments to ensure both a large sample size and a high diapause incidence to reduce errors caused by genetic drift. The low temperatures used to induce diapause in the ld and the wt strains were 20 and 23 °C, respectively. To facilitate comparisons, the temperatures used for each crossing were the same as used for the nd parental strain.

The diapause incidence was determined 35 days after larviposition by removing the caps of the puparia to observe the developmental status of the pupae, using criteria previously described (Fraenkel and Hsiao 1968). After assessing the diapause incidences of F1, F2, BcN, BcD, and each parental strain, an exact test was performed to determine whether there was a specific coherent incidence ratio within each crossing line (Martin and Austin 1996).

Results

The recorded diapause incidences are shown in Table 1. The 2 parallel sets of crossings yielded a similar pattern. First, the diapause incidences were nearly the same in both directions of crossing, suggesting that genetically based differences in diapause induction between the strains do not involve sex linkage. Second, the nd phenotype was an almost completely dominant trait as evidenced by the lack of diapause (nd × ld) or extremely low diapause (ld) incidence (nd × wt) in the F1 generation. Third, the diapause incidence in the F2 generation was roughly a quarter of that in the diapausing strain, implying a 3:1 Mendelian segregation ratio. Finally, the backcrossing results showing no diapasing flies in the backcross BcN and a diapause incidence reduced by half in BcD, further indicated that inheritance of diapause operates in a simple Mendelian manner. Collectively, these results are consistent with a Mendelian inheritance model in which the nondiapause phenotype in the nd strain is a single autosomal dominant trait.

### Table 1. Diapause incidences of progeny from reciprocal crossing between the nondiapausing strain (N) and the 2 diapausing strains (D) of *Sarcophaga bullata*, designated low diapause (ld) and wild type (wt). The strain of the female parent is designated on the left. Parentheses indicate hybrids resulting from each specific crossing.

<table>
<thead>
<tr>
<th>Strain/hybrid</th>
<th>nd × ld</th>
<th>Total number</th>
<th>Diapause incidence (%)</th>
<th>Total number</th>
<th>Diapause incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P D</td>
<td>28.4</td>
<td>229</td>
<td>78.1</td>
<td>811</td>
<td>8.7</td>
</tr>
<tr>
<td>N</td>
<td>0.0</td>
<td>262</td>
<td>0.0</td>
<td>768</td>
<td>0.0</td>
</tr>
<tr>
<td>F1 N × D</td>
<td>0.0</td>
<td>277</td>
<td>1.0</td>
<td>415</td>
<td>0.0</td>
</tr>
<tr>
<td>D × N</td>
<td>0.0</td>
<td>272</td>
<td>0.6</td>
<td>311</td>
<td>0.0</td>
</tr>
<tr>
<td>F2 (N × D) × (N × D)</td>
<td>8.7</td>
<td>321</td>
<td>15.5</td>
<td>736</td>
<td>3.6</td>
</tr>
<tr>
<td>(D × N) × (D × N)</td>
<td>3.6</td>
<td>252</td>
<td>21.6</td>
<td>693</td>
<td>8.6</td>
</tr>
<tr>
<td>BcD D × (N × D)</td>
<td>14.8</td>
<td>54</td>
<td>41.7</td>
<td>412</td>
<td>14.8</td>
</tr>
<tr>
<td>(N × D) × D</td>
<td>8.6</td>
<td>35</td>
<td>47.9</td>
<td>1275</td>
<td>9.1</td>
</tr>
<tr>
<td>(D × D) × N</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>233</td>
<td>0.0</td>
</tr>
<tr>
<td>(D × N) × D</td>
<td>—</td>
<td>—</td>
<td>53.1</td>
<td>653</td>
<td>0.0</td>
</tr>
<tr>
<td>BcN N × (N × D)</td>
<td>0.0</td>
<td>174</td>
<td>0.3</td>
<td>310</td>
<td>0.0</td>
</tr>
<tr>
<td>(N × D) × N</td>
<td>0.0</td>
<td>272</td>
<td>0.0</td>
<td>278</td>
<td>0.0</td>
</tr>
<tr>
<td>N × (D × N)</td>
<td>0.0</td>
<td>33</td>
<td>0.0</td>
<td>132</td>
<td>0.0</td>
</tr>
<tr>
<td>(D × N) × N</td>
<td>0.0</td>
<td>13</td>
<td>0.0</td>
<td>216</td>
<td>0.0</td>
</tr>
</tbody>
</table>
represent observed diapause incidences. The ratio of cumulative diapause incidences in the non-diapausing strain (nd) and the diapausing parent (D) was close to 0:0:1:2:4 (P > 0.05, exact test), as predicted by the model. In both crosses, the nd characteristc in the nd strain was traced to one gene locus, based on the fact that diapause induction in flesh flies is inherited in a Mendelian manner. Therefore, it is concluded that a single gene or a cluster of genes arranged closely on the same chromosome completely controls pupal diapause in S. bullata.

Discussion

Diapause is a seasonal response programed by the central photoperiodic clock (Saunders and Sutton 1969), using integrated information of photoperiod and temperature (Christiansen-Weniger and Hardie 1999). Studying the inheritance mode of diapause provides an indirect way to investigate the regulation of diapause induction and may help to decipher mechanisms of the photoperiodic clock.

In some species such as the parasitic wasp, Nasonia vitripennis, genetic factors affecting the diapause response are mainly cytoplasmic, as implicated by the purely maternal role of diapause determination (Saunders 1965). However, in most cases, these determinants are directly derived from genomic information. Moreover, diapause can be monogenetically controlled in some species. For example, mutations on a single photoreceptor gene are sufficient to affect the photoperiodic response of diapause in the spider mite, Tetranychus urticae (Veerman 1980). Finding these single diapause-controlling genes and studying their influence on diapause is a promising way to help construct networks of unknown genes involved in the photoperiodic clock system.

The diapause-controlling genes are not necessarily homologous in various species, as suggested by different modes of inheritance discovered so far. Even between very closely related species, the identities of diapause-controlling genes may differ. For instance, a diapause-controlling gene is on an autosome in Drosophila littoralis but on the X chromosome in Drosophila lummei, thus suggesting a role for different genes given that gene translocation is unlikely to occur between these 2 interbreeding species (Lumme and Oikarinen 1977; Lumme and Keranen 1978). It is reasonable to hypothesize that different genes play essential roles at different levels of the complex photoperiodic machinery. Thus, we can expect that a number of different genes are involved in the diapause response but that a mutation in any one of these genes may result in a loss of the diapause phenotype.

It has been suggested that a small number of genes play essential roles in pupal diapause determination in S. bullata, a conclusion derived from crossing 2 lines selected for a high and an ld incidence (Henrich and Denlinger 1983). Our current study involved crosses from a recently collected wild strain of S. bullata and 2 laboratory lines, one that exhibited no diapause and another line that exhibited an ld incidence. In the present study, we discovered the existence of only one single genetic unit that completely controls diapause in this species. An abnormality on a single autosomal gene, most likely due to a mutation, deprives the flies of the capacity to enter pupal diapause, despite the presence of strong diapause-inducing conditions. This abnormal nd allele is dominant to the wt allele. In other words, a single gene or closely linked gene cluster, when altered, completely shuts down the photoperiodic induction of diapause. The locus is so essential for the proper functioning of the photoperiodic clock that almost all individual flies are prevented from entering diapause as long as the nd allele is present, even under a heterozygous condition. This gene is thus a major diapause-controlling gene that regulates entry into diapause.

No noticeable morphological or developmental aberrations are exhibited by the nd strain of S. bullata, but we suspect that this gene is involved in the circadian clock system. In contrast to the 2 diapausing strains that exhibit distinct daily eclosion rhythms, the nd strain of S. bullata both lacks the circadian rhythmicity of adult eclosion and the photoperiodic response of diapause (Goto et al. 2006). The flies
emerge throughout the day, in contrast to wt flies that show an eclosion peak at dawn (Saunders 2002). Therefore, this diapause-controlling gene is likely to be responsible for the loss of circadian timing as well. The involvement of this single diapause-controlling gene in circadian clock function is supported by the significant transcriptional elevation and reduced daily oscillation of 2 major circadian clock genes, *period* and *timeless* (Goto et al. 2006).

Although there are some suggestions that circadian and photoperiodic systems may act independently (e.g., Dolezel et al. 2005; Lankinen and Forsman 2006), there are also numerous reports suggesting that these 2 systems are functionally linked, as evidenced by correlations between transcription of circadian clock genes and photoperiodic responses (Goto and Denlinger 2002; Syrova et al. 2003). Evidence suggesting that molecular components are shared between these 2 time measuring systems was also obtained in experiments with other species such as the drosophilid fly, *Chymomyza costata* (Kostal and Shimada 2001; Pavelka et al. 2003) and *Drosophila melanogaster* (Tauber et al. 2007).

Central circadian clock genes including *period* (Konopka and Benzer 1971), *timeless* (Sehgal et al. 1994), *cycle* (Rutila et al. 1998), *clock* (Allada et al. 1998; Darlington et al. 1998) as well as the circadian photoreceptor *cryptochrome* (Stanewsky et al. 1998) are all candidate genes that could potentially be responsible for loss of the diapause response. A survey for functional mutations in these candidate genes in the nd strain might suggest the basis for this nd phenotype, but it is also quite possible that the gene represents a novel photoperiodic clock gene or an upstream or downstream gene that is essential for the proper functioning of clock input or output mechanisms.

**Funding**

National Science Foundation (IOB-0416720).

**Acknowledgments**

We appreciate thoughtful reviews of the manuscript provided by Dr Vincent C. Henrich, University of North Carolina, Greensboro, and 2 anonymous reviewers.

**References**


Mori A, Romero-Severson J, Severson DW. 2007. Genetic basis for reproductive diapause is correlated with life history traits within the *Culex pipiens* complex. Insect Mol Biol. 16:515–524.


Received May 12, 2008; Revised August 10, 2008; Accepted September 5, 2008

Corresponding Editor: James Thompson