Journal of Heredity 2009:100(2):251–255
doi:10.1093/jhered/esn082
Advance Access publication October 3, 2008

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 Sternburg 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, *Chlorops 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 puerariicola* (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

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.

Strain/hybrid		nd × ld		nd × wt	
		Diapause incidence (%)	Total number	Diapause incidence (%)	Total number
P	D	28.4	229	78.1	811
	N	0.0	262	0.0	768
F_1	N × D	0.0	277	1.0	415
	D × N	0.0	272	0.6	311
F_2	(N × D) × (N × D)	8.7	321	15.5	736
	(D × N) × (D × N)	3.6	252	21.6	693
BcD	D × (N × D)	14.8	54	41.7	412
	(N × D) × D	8.6	35	47.9	1275
	D × (D × N)	—	—	41.2	233
	(D × N) × D	—	—	53.1	653
BcN	N × (N × D)	0.0	174	0.3	310
	(N × D) × N	0.0	272	0.0	278
	N × (D × N)	0.0	33	0.0	132
	(D × N) × N	0.0	13	0.0	216

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°N latitude) was used. Stocks of all these colonies were reared throughout development in long-day photoperiod conditions (light:dark 15:9 h) at 25 °C.

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 (F_1) and second generation resulting from self-crossing of F1 flies (F_2) 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 F_1, F_2, 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 F_1 generation. Third, the diapause incidence in the F_2 generation was roughly a quarter of that in the diapausing strain, implying a 3:1 Mendelian segregation ratio. Finally, the backcrossing results showing no diapausing 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.

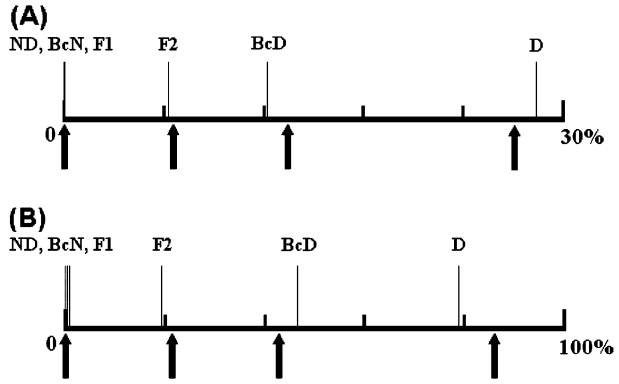

Figure 1. Comparisons of diapause incidences of *Sarcophaga bullata* with theoretically predicted values by a Mendelian inheritance model in 2 crosses: nd × ld (**A**) and nd × wt (**B**). nd: nondiapausing strain, ld: strain with an ld incidence, wt: wild-type strain with a high diapause incidence. The 4 arrows (from left to right) point out the theoretically predicted diapause incidences of N (nd strain), F_2, BcD, D (diapausing strain: ld in **A** and wt in **B**), respectively. BcN and F_1 share the same predicted diapause incidences as N. Vertical lines represent observed diapause incidences.

According to the hypothesized Mendelian inheritance model, the diapause incidences in these crossing results should exhibit 1) no sex biases and 2) simple numeric relationships. The lack of sex biases was tested by comparing the diapause incidences between the 2 directions in the F_1 and F_2 hybrids as well as among the 4 directions in the backcrosses. Because no significant differences were found ($P > 0.05$, exact test), cumulative diapause incidences of F_1, F_2, and backcrosses were calculated. As shown in Figure 1, the relationships among recorded diapause incidences also supported this model, which was constructed by compiling all the observed data in a "least-error" way. The ratio of cumulative diapause incidences in the nondiapausing (nd) parent (N), BcN, F_1, F_2, BcD, and the diapausing parent (D) was close to 0:0:0:1:2:4 ($P > 0.05$, exact test), as predicted by the model. In both crosses, the nd characteristic 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 2 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 wt 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 aberrances 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 may 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

Allada R, White N, So WV, Hall JC, Rosbash M. 1998. A mutant *Drosophila* homolog of mammalian *Clock* disrupts circadian rhythms and transcription of *period* and *timeless*. Cell. 93:791–804.

Christiansen-Weniger P, Hardie J. 1999. Environmental and physiological factors for diapause induction and termination in the aphid parasitoid, *Aphidius ervi* (Hymenoptera: Aphidiidae). J Insect Physiol. 45:357–364.

Danks HV. 1987. Insect dormancy: an ecological perspective. Ottawa (Canada): Biological Survey of Canada.

Darlington TK, Wager-Smith K, Ceriani MF, Staknis D, Gekakis N, Steeves DL, Weitz CJ, Takahashi JS, Kay SA. 1998. Closing the circadian loop: CLOCK-induced transcription of its own inhibitors *per* and *tim*. Science. 280:1599–1602.

Denlinger DL. 1971. Embryonic determination of pupal diapause in the flesh fly, *Sarcophaga crassipalpis* Macquart. J Insect Physiol. 17:1815–1822.

Denlinger DL. 1972. Induction and termination of pupal diapause in *Sarcophaga* (Diptera: Sarcophagidae). Biol Bull. 142:11–24.

Denlinger DL. 2002. Regulation of diapause. Annu Rev Entomol. 47:93–122.

Dolezel D, Vaneckova H, Sauman I, Hodkova M. 2005. Is *period* gene causally involved in the photoperiodic regulation of reproductive diapause in the linden bug, *Pyrrhocoris apterus*? J Insect Physiol. 51:655–659.

Fraenkel G, Hsiao C. 1968. Morphological and endocrinological aspects of pupal diapause in a flesh fly, *Sarcophaga argyrostoma* and *S. bullata*. J Insect Physiol. 14:689–705.

Gnagey AL, Denlinger DL. 1984. Photoperiodic induction of pupal diapause in the flesh fly, *Sarcophaga crassipalpis*: embryonic sensitivity. J Comp Physiol. 154:91–96.

Goto SG, Han B, Denlinger DL. 2006. A nondiapausing variant of the flesh fly, *Sarcophaga bullata*, that shows arrhythmic adult eclosion and elevated expression of two circadian clock genes, *period* and *timeless*. J Insect Physiol. 52:1213–1218.

Goto SG, Denlinger DL. 2002. Short-day and long-day expression patterns of genes involved in the flesh fly clock mechanism: period, timeless, cycle, and cryptochrome. J Insect Physiol. 48:803–816.

Hayes JL, Bandy DJ, Dingle H. 1987. Genetic analysis of diapause differences between populations of the large milkweed bug. J Hered. 78:266–270.

Henrich VC, Denlinger DL. 1982. A maternal effect that eliminates pupal diapause in progeny of the flesh fly, *Sarcophaga bullata*. J Insect Physiol. 28:881–884.

Henrich VC, Denlinger DL. 1983. Genetic differences in pupal diapause incidence between two selected strains of the flesh fly. J Hered. 74:371–374.

Kim Y, Krafsurk ES, Bailey TB, Zhao S. 1995. Mode of inheritance of face fly diapause and its correlation with other developmental traits. Ecol Entomol. 20:359–366.

Konopka RJ, Benzer S. 1971. Clock mutants of *Drosophila melanogaster*. Proc Natl Acad Sci USA. 68:2112–2116.

Kostal V, Shimada K. 2001. Malfunction of circadian clock in the *non-photoperiodic-diapause* mutants of the drosophilid fly, *Chymomyza costata*. J Insect Physiol. 47:1269–1274.

Lankinen P, Forsman P. 2006. Independence of genetic geographical variation between photoperiodic diapause, circadian eclosion rhythm, and Thr-Gly repeat region of the *period* gene in *Drosophila littoralis*. J Biol Rhythms. 21:3–12.

Lee KY, Valaitis AP, Denlinger DL. 1997. Future evidence that diapause in gypsy moth, *Lymantria dispar*, is regulated by ecdysteroids: a comparison of diapause and nondiapause strains. J Insect Physiol. 43:897–903.

Lumme J, Keranen L. 1978. Photoperiodic diapause in *Drosophila lummei* Hackman is controlled by an X-chromosomal factor. Hereditas. 89:261–262.

Lumme J, Oikarinen A. 1977. The genetic basis of the geographically variable photoperiodic diapause in *Drosophila littoralis*. Hereditas. 86:129–142.

Martin DO, Austin H. 1996. Exact estimate for a rate ratio. Epidemiology. 7:29–33.

McWatters HG, Saunders DS. 1996. The influence of each parent and geographic origin on larval diapause in the blow fly, *Calliphora vicina*. J Insect Physiol. 42:721–726.

McWatters HG, Saunders DS. 1997. Inheritance of the photoperiodic response controlling larval diapause in the blow fly, *Calliphora vicina*. J Insect Physiol. 43:709–717.

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.

Pavelka J, Shimada K, Kostal V. 2003. TIMELESS: a link between fly's circadian and photoperiodic clocks? Eur J Entomol. 100:255–265.

Reed GL, Guthrie WD, Showers WB, Barry BD, Cox DF. 1981. Sex linked inheritance of diapause in the European corn borer *Ostrinia nubilalis*, its significance to diapause physiology and environmental response of the insect. Ann Entomol Soc Am. 74:1–8.

Riihimaa A, Kimura M, Lumme J, Lakovaara S. 1996. Geographical variation in the larval diapause of *Chymomyza costata* (Diptera; Drosophilidae). Hereditas. 124:151–164.

Roff DA, Bradford MJ. 2000. A quantitative genetic analysis of phenotypic plasticity of diapause induction in the cricket *Allonemobius socius*. Heredity. 84:193–200.

Rutila JE, Suri V, Le M, So V, Rosbash M, Hall JC. 1998. CYCLE is a second bHLH-PAS clock protein essential for circadian rhythmicity and transcription of *Drosophila period* and *timeless*. Cell. 93:805–814.

Saunders DS. 1965. Larval diapause of maternal origin: induction of diapause in *Nasonia vitripennis* (Walk) (Hymenoptera: Pteromalidae). J Exp Biol. 42:495–508.

Saunders DS. 2002. Insect clocks. Amsterdam (The Netherlands): Elsevier Science.

Saunders DS, Sutton D. 1969. Circadian rhythms in the insect photoperiodic clock. Nature. 221:559–561.

Sehgal A, Price JC, Man B, Young MW. 1994. Loss of circadian behavioral rhythms and *per* mRNA oscillations in the *Drosophila* mutant *timeless*. Science. 263:1603–1606.

Stanewsky R, Kaneko M, Emery P, Beretta B, Wager-Smith K, Kay SA, Rosbash M, Hall JC. 1998. The *cry*^{b} mutation identifies cryptochrome as a circadian photoreceptor in *Drosophila*. Cell. 95:681–692.

Suwa A, Gotoh T. 2006. Geographic variation in diapause induction and mode of diapause inheritance in *Tetranychus puerariicola*. J Appl Entomol. 130:329–335.

Syrova Z, Dolezel D, Saumann I, Hodkova M. 2003. Photoperiodic regulation of diapause in linden bugs: are *period* and *Clock* genes involved? Cell Mol Life Sci. 60:2510–2515.

Takeda M. 1998. Genetic basis of photoperiodic control of summer and winter diapause in geographic ecotypes of the rice stem maggot, *Chlorops oryzae*. Entomol Exp Appl. 86:59–70.

Tauber E, Zordan M, Sandrelli F, Pegoraro M, Osterwalder N, Breda C, Daga A, Selmin A, Monger K, Benna C, et al. 2007. Natural selection favors a newly derived *timeless* allele in *Drosophila melanogaster*. Science. 316: 1895–1898.

Tauber MJ, Tauber CA, Masaki S. 1986. Seasonal adaptations of insects. Oxford: Oxford University Press.

Veerman A. 1980. Functional involvement of carotenoids in photoperiodic induction of diapause in the spider mite, *Tetranychus urticae*. Physiol Entomol. 5:291–300.

Waldbauer GP, Sternburg JG. 1973. Polymorphic termination of diapause by *Cecropia*: genetic and geographical aspects. Biol Bull. 145:627–641.

Wipking W, Kurtz J. 2000. Genetic variability in the diapause response of the burnet moth *Zygaena trifolii* (Lepidoptera: Zygaenidae). J Insect Physiol. 46:127–134.

Yamashita O. 1996. Diapause hormone of the silkworm, *Bombyx mori*: structure, gene expression and function. J Insect Physiol. 42:669–679.

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

Corresponding Editor: James Thompson