J Comp Physiol A (2009) 195:825–829
DOI 10.1007/s00359-009-0460-5

ORIGINAL PAPER

Environmental control of ovarian dormancy in natural populations of *Drosophila melanogaster*

Kevin J. Emerson · Alison M. Uyemura · Keely L. McDaniel · Paul S. Schmidt · William E. Bradshaw · Christina M. Holzapfel

Received: 16 March 2009 / Revised: 3 July 2009 / Accepted: 9 July 2009 / Published online: 9 August 2009

Abstract *Drosophila melanogaster* from Australia, Europe and North America enter an adult ovarian dormancy in response to short days and low temperatures. The independent effects of temperature and day length in the determination of dormancy have been examined only in one long-established laboratory line (Canton-S). In all other studies of natural or laboratory populations, dormancy has been assessed at either a single short day or a single moderately low temperature. Herein, we determine the relative roles of temperature, photoperiod, and their interaction in the control of ovarian dormancy in *D. melanogaster* from two natural populations representing latitudinal extremes in eastern North America (Florida at 27°N and Maine at 44°N). In both natural populations, temperature is the main determinant of dormancy, alone explaining 67% of the total variation among replicate isofemale lines, whereas photoperiod has no significant effect. We conclude that ovarian dormancy in *D. melanogaster* is a temperature-initiated syndrome of winter-tolerant traits that represents an adaptive phenotypic plasticity in temperate seasonal environments.

Keywords Photoperiodism · Geographic variation · Diapause · Stress resistance · Life-history

K. J. Emerson (✉) · A. M. Uyemura · K. L. McDaniel · W. E. Bradshaw · C. M. Holzapfel
Center for Ecology and Evolutionary Biology,
5289 University of Oregon, Eugene, OR 97403-5289, USA
e-mail: kemerson@uoregon.edu

P. S. Schmidt
Department of Biology, University of Pennsylvania,
Philadelphia, PA 19104, USA

Abbreviation

L:D Number of hours of light (L) and dark (D) in a given environmental cycle

Introduction

All organisms live in seasonal environments and exhibit life histories that permit the reduction or mitigation of the effects of unfavorable seasons. In temperate regions, photoperiodic (day length) cues are a highly reliable predictor of the seasons and are used by many temperate arthropods to time the onset of dormancy in concert with the onset of winter (Tauber et al. 1986; Danks 1987; Leather et al. 1993). Photoperiodic cues are much weaker at lower latitudes, where arthropods tend to use other cues, such as temperature or humidity, to time seasonal dormancy (Denlinger 1986). *Drosophila melanogaster* originated in tropical Africa and has been introduced into temperate North America over the last 400 years (David and Capy 1988). In adult females, the combined effects of short days and low temperatures induce ovarian dormancy (Saunders et al. 1989; Saunders and Gilbert 1990; Williams and Sokolowski 1993; Tatar et al. 2001; Williams et al. 2006). However, the separate effects of day length and temperature have only been determined in a single long-established laboratory line, Canton-S (isolated from Ohio ~1930, Bridges and Brehme 1944). When temperature and day length are varied independently, longer days and warmer temperatures inhibit and shorter days and cooler temperatures promote ovarian dormancy in Canton-S females (Saunders and Gilbert 1990). To date, the effect of photoperiod in natural populations of *D. melanogaster* has

been evaluated only in European populations and then only at a single temperature (Tauber et al. 2007). Herein, we examine the relative roles of photoperiod and temperature in determining ovarian dormancy in natural populations that reflect extreme latitudinal ranges of *D. melanogaster* from eastern North America.

Methods

Fly stocks

We sampled 12 isofemale lines on strawberries from Watch Me Grow Farms in Ft. Pierce, FL (27° 28′ N, 80° 21′ W) in April, 2007 and ten isofemale lines from apples at Rocky Ridge Orchard in Bowdoinham, ME (44° 02′ N, 69° 52′ W) in October 2006, representing nearly 20° of latitudinal difference. Isofemale lines were established in the field and subsequently maintained in the laboratory under Light:Dark = L:D = 12:12 at 22–24°C prior to the experiments. All experiments were completed within 19 months of the collection of the lines.

Throughout the experiments described below, flies were reared in 4.2 × 10 cm (diameter × height) vials containing 4 g of Formula 4-24 Instant Drosophila Medium (Carolina Biological Supply), 150 mg of Fleishman's active dry yeast, and 10 mL of nanopure water per vial. Flies were transferred to fresh vials weekly or more often as needed.

Experimental chambers

Experimental chambers measured 53 × 49 × 18 cm (width × depth × height) with bright white interiors. Light in the chambers was provided by white-light, nine-LED strips that yield a full spectrum of visible light (Sylvania LED/UC/W/9W) creating only 0.2 W of heat. LED strip lights were used as a light source because, unlike traditional incubator lights, LEDs produce negligible heat. Heat production by lights is a concern when testing for day length by temperature interaction where more total heat is produced by the lights during longer days and may falsely enhance the apparent effect of day length on development. Each chamber was cooled by air blown through light-baffled ducts. Two blocks of eight chambers were housed in each of two low temperature incubators but response to long and short days was always assessed within the same incubator. The insides of the incubators were maintained in darkness and were opened only when all experimental chambers were in the light phase of the L:D cycle.

To confirm that Maine and Florida flies were responsive to experimental lighting, we placed a vial of flies in the darkest corner of the chamber and noted universal, positive phototaxis within 10 s, both with all LEDs exposed and with eight of the nine LEDs covered with opaque electrical tape. In response to a reviewer's comment we later confirmed that flies from the Canton-S strain were equally positively phototactic using the same protocol as above. As a further positive control for the efficacy of the LEDs to elicit a photoperiodic response, we exposed 90+ diapausing larvae of the mosquito *Wyeomyia smithii* to long and short days at 25°C with nine or only one LED exposed. Long days elicited 92 and 94% development and short days elicited 0 and 1% development in response to nine or one LED, respectively. Hence, either all nine or a single LED was sufficient to elicit a full photoperiodic response in a mosquito known to be sensitive to day length (Bradshaw and Lounibos 1977).

Scoring ovarian development

We collected flies under stock rearing conditions and sorted them into cohorts with 5 males and 20 females each, with sex determined by the presence or absence of sex combs under carbon dioxide anesthesia. Cohorts were placed in experimental conditions within 3 h of adult eclosion. Since flies are sensitive to temperature and incubator thermometers are unreliable indicators of temperatures actually experienced by flies, all temperature data were recorded in each of the eight experimental treatments by three replicate 15 × 5 mm (diameter × height) data loggers (Watchdog Data Loggers, 100 series, Spectrum Technologies) held within fly vials. At the end of the experimental period, we dissected all the females in each cohort and scored them for ovarian development. Reproductive dormancy was defined following King (1970): Dormancy was defined as the presences of only stage 1–7 ovarioles in both ovaries; development was defined as at least one stage 8 ovariole in either ovary (Schmidt et al. 2005a, b; Schmidt and Conde 2006).

Experimental protocol

Replicated isofemale lines from Maine and Florida were used to determine the effects of temperature, photoperiod and latitude (population) on ovarian dormancy in disjunct natural populations of *D. melanogaster*. Each cohort was placed into one of eight environmental treatments made up of either long days (Light: Dark = L:D = 18:06) or short days (L:D = 10:14) at 10, 11, 12 or 14°C (Fig. 1). We chose these temperatures to span those used in previous studies of dormancy in *D. melanogaster* (Saunders et al. 1989; Tatar et al. 2001; Williams et al. 2006; Tauber et al. 2007). Two to three replicate cohorts for each isofemale line were initiated for each of the eight treatments. Flies were left in each treatment for 25 (11, 12, and 14°C treatments) or 28 (10°C treatment) days, at which point the

animals were frozen, dissected, and scored for ovarian development. We scored an average of 41 ± 10 (mean ± SD) females for reproductive dormancy in each of the 22 lines in each of the 8 temperature × photoperiod treatments. A total of 8,551 flies were scored for this experiment.

Data analysis

The percentage of females expressing reproductive dormancy (arcsin-square root transformed) was modeled as a function of population (Pop), photoperiod (PPD), temperature (Temp) and isofemale line nested within population [Line (Pop)] and all of the associated interaction terms. The mean square for Line (Pop) was used as the mean square error term for all F tests that included only the other effects, as the lines were the independent measurements under these conditions. The residual mean square (representing variation among replicate cohorts within lines) served as the mean square error term for all effects including Line (Pop). All statistical analyses were performed using the R program for statistical computing (R Development Core Team 2007).

Results

Temperature was the primary determinant of dormancy in natural lines of *D. melanogaster* from both Florida and Maine (Fig. 2), alone explaining 67% of the total variation (Table 1). Note that in the 10°C treatment, cohorts had three more days to develop than at higher temperatures so the differences between the 10°C and the other treatments are, if anything, underestimates. Population of origin, photoperiod (Fig. 2), or their individual or combined interaction with temperature (Table 1) had no significant overall effect and, together, explained only 2.4% of the total variation in dormancy.

Consistent with previous work on the incidence of diapause in North American *D. melanogaster* (Schmidt et al. 2005a; Williams and Sokolowski 1993), the northern population has a higher percentage of ovarian dormancy than the southern population under short day lengths at 12°C (Tukey's HSD, $P < 0.001$) (Fig. 2). There was a significant effect of isofemale line (nested within population) and isofemale line by temperature interaction, but no significant effect of line by photoperiod, temperature by photoperiod or their three-way interaction (Table 1). Hence, there was greater variation in response to temperature within than between populations and photoperiod had no significant effect either between populations or among lines within populations.

Discussion

In natural populations of *D. melanogaster* from highly disjunct populations in eastern North America, temperature is a far more important determinant of ovarian dormancy than day length or population of origin (Fig. 2). The control of dormancy in natural populations of *D. melanogaster* in temperate eastern North America resembles that of tropical insects where temperature and not photoperiod is the major factor initiating dormancy (Denlinger 1986). *Drosophila melanogaster* in eastern North America therefore retains the more tropical control of dormancy, even in a high-latitude population (Maine). This result is in contrast to European *D. melanogaster*, which have been shown to be photoperiodic at 13°C (Tauber et al. 2007). There is also variation in the thermal threshold for the induction of

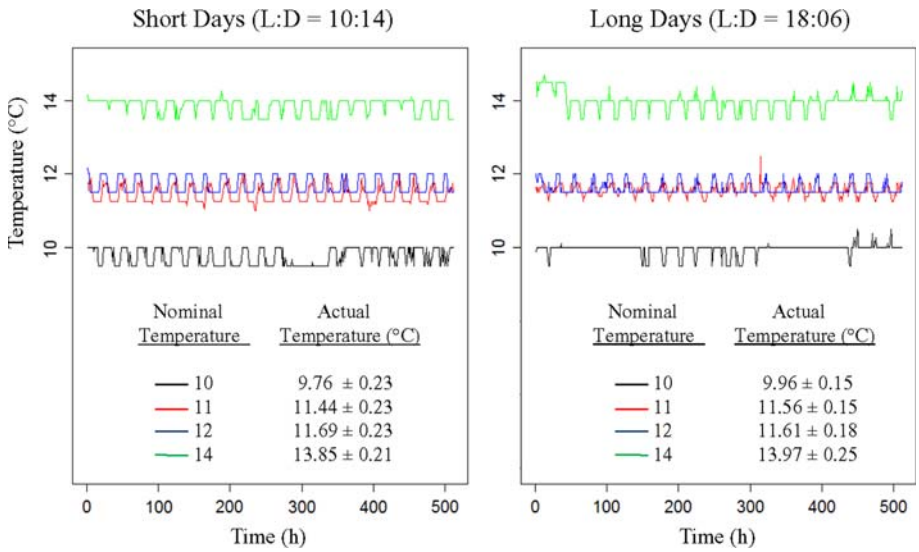

Fig. 1 Representative thermal profiles for each of the four thermal treatments under both long and short day lengths. Profiles are the average of two to three independent data loggers (Watchdog 100, Spectrum Technologies, Inc.) in the fly vials themselves for each of the eight light and temperature combinations in the study. The *inset* shows the actual mean ± standard deviation temperature for each of the nominal temperature treatments

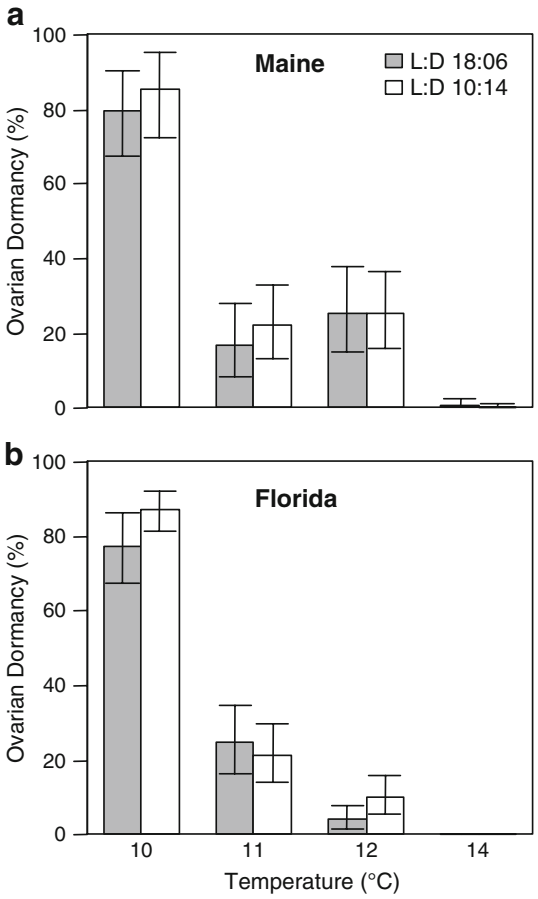

Fig. 2 Percentage of individuals expressing ovarian dormancy in natural populations of *D. melanogaster* from **a** Maine and **b** Florida in response to temperature (Table 1) and day length (L:D = hours Light: hours Dark). Temperature is the primary determinant of ovarian dormancy. *Error bars* represent mean ± two standard errors on a percentage scale, backtransformed from data analyzed after arcsin-square root transformation (Table 1) and represent variation among lines within treatments

Table 1 ANOVA table for the effects of experimental treatments on the incidence of ovarian dormancy (arcsin-square root transformed) within and between natural populations of *D. melanogaster* from Maine and Florida

Source of variation	*Df*	SS	MS	*F*	*P*
Temp	3	46.824	15.608	70.062[a]	**<0.001**
PPD	1	0.114	0.114	0.511[a]	0.483
Pop	1	0.423	0.423	1.898[a]	0.183
Temp × PPD	3	0.164	0.055	0.246[a]	0.863
Temp × Pop	3	0.883	0.294	1.321[a]	0.295
PPD × Pop	1	0.013	0.013	0.056[a]	0.815
Temp × Pop × PPD	3	0.098	0.033	0.147[a]	0.931
Line (Pop)	20	4.455	0.223	6.170[b]	**<0.001**
Temp × line (Pop)	60	3.858	0.067	1.842[b]	**<0.001**
PPD × line (Pop)	20	0.677	0.034	0.937[b]	0.540
Temp × PPD × line (Pop)	60	1.225	0.021	0.585[b]	0.993
Cohorts = residuals	327	11.806	0.036		

Significant effects have bold P-values

Temp temperature, *PPD* photoperiod, *Pop* population, *Line* isofemale line, *Df* degrees of freedom, *SS* sum of squares, *MS* mean squares, *F* F-statistic

[a] Error mean square based on lines within populations

[b] Variation within populations; isofemale lines nested within populations; error mean square based on cohorts within lines

dormancy between North America and Europe: European flies show ~50% induction of dormancy at 13°C under a range of day lengths (Tauber et al. 2007), whereas North American flies do not reach 50% induction of dormancy until temperature falls to 10–12°C.

In a Canton-S laboratory line of *D. melanogaster* from North America, day length has an effect on ovarian dormancy, but only over a narrow range of temperatures (Saunders and Gilbert 1990). In their study, temperature had a larger effect that photoperiod, with the induction of dormancy increasing from <10% at 13°C to >70% at 10°C. This narrow range of temperature contrasts dramatically with ovarian dormancy in Asian flies in the *D. auraria* complex (Kimura 1984) and European *D. littoralis* (Lankinen 1986) that are photoperiodic over a wider range of higher temperatures. In order to express dormancy reliably, Canton-S flies must be transferred from warmer to diapause-inducing low temperatures within a few hours of adult eclosion (Saunders and Gilbert 1990) and ovarian development resumes in Canton-S within 8–12 h after transfer from diapause-inducing to room temperature (Richard et al. 1998). We have shown that even in natural populations of North American *D. melanogaster*, a decrease in temperature from 12 to 10°C results in an increase in ovarian dormancy from 20 to 80%. Hence, a 2°C change in temperature experienced by eastern North American populations elicits approximately the same response in the incidence of diapause as a 10 h change in day length among European populations (Tauber et al. 2007). Though photoperiod may affect as yet undetermined physiological processes in *D. melanogaster*, it is unlikely that it plays a substantive ecological role in the regulation of ovarian dormancy in nature where temperatures are not constant but are highly variable.

In eastern North American populations of *D. melanogaster*, selection in stressful laboratory environments has resulted in increased incidence of ovarian dormancy, increased cold resistance, and increased lipid content, but also a tradeoff of reduced fecundity early in adult life (Schmidt and Conde 2006). In rural orchards, the genetic propensity to enter ovarian dormancy is high in the spring and declines during the summer and early fall (Schmidt and Conde 2006). When lines of *D. melanogaster* are selected

for early and late reproduction and then grown in outdoor cages in eastern Australia, the late selected lines achieve higher fecundity in the spring and, hence, higher overwintering fitness than early selected lines (Hoffmann et al. 2003). These experiments indicate that ovarian dormancy represents a genetically variable phenotypic plasticity that is under seasonal selection in nature. We conclude that in *D. melanogaster*, low temperatures herald the imminent approach of winter and are the primary environmental factors that induce a syndrome of traits likely to be co-adapted for overwintering: ovarian dormancy, high lipid stores and greater tolerance of environmental stress, especially cold.

Acknowledgments We thank V. Koštál, and E. Tauber for discussion and for comments on previous versions of this paper, and L. Sherson and K. Kim for participation in the early stages of this research. J. Giebultowicz graciously supplied our Canton-S line. All work presented here complied with the "Principles of animal care", publication No. 86-23 of the National Institute of Health, and also with current laws of the United States, where these experiment were performed. This work was made possible by generous support from the National Science Foundation through grants DEB-0412573 and IOB-0445710 to WEB, grant DEB-0542859 to PSS, and the National Science Foundation IGERT Training program grant DGE-0504727 to KJE.

References

Bradshaw WE, Lounibos LP (1977) Evolution of dormancy and its photoperiodic control in pitcher-plant mosquitoes. Evolution 31:546–567

Bridges CB, Brehme KS (1944) The mutants of *Drosophila melanogaster*. Carnegie Institution of Washington, Washington

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

David JR, Capy P (1988) Genetic variation of *Drosophila melanogaster* natural populations. Trends Genet 4:106–111

Denlinger DL (1986) Dormancy in tropical insects. Annu Rev Entomol 31:239–264

Hoffmann AA, Scott M, Partridge L, Hallas R (2003) Overwintering in *Drosophila melanogaster*: outdoor field cage experiments on clinal and laboratory selected populations help to elucidate traits under selection. J Evol Biol 16:614–623

Kimura MT (1984) Geographic variation of reproductive diapause in the *Drosophila auraria* complex (Diptera, Drosophilidae). Physiol Entomol 9:425–431

King RC (1970) Ovarian development in *Drosophila melanogaster*. Academic Press, New York

Lankinen P (1986) Geographical variation in circadian eclosion rhythm and photoperiodic adult diapause in *Drosophila littoralis*. J Comp Phys A 159:123–142

Leather SR, Walters KFA, Bale JS (1993) The ecology of insect overwintering. Cambridge University Press, Cambridge

R Development Core Team (2007) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Richard DS, Watkins NL, Serafin RB, Gilbert LI (1998) Ecdysteroids regulate yolk protein uptake by *Drosophila melanogaster* oocytes. J Insect Physiol 44:637–644

Saunders DS, Gilbert LI (1990) Regulation of ovarian diapause in *Drosophila melanogaster* by photoperiod and moderately low temperature. J Insect Physiol 36:195–200

Saunders DS, Henrich VC, Gilbert LI (1989) Induction of diapause in *Drosophila melanogaster*: photoperiodic regulation and the impact of arrhythmic clock mutations on time measurement. Proc Natl Acad Sci USA 86:3748–3752

Schmidt PS, Conde DR (2006) Environmental heterogeneity and the maintenance of genetic variation for reproductive diapause in *Drosophila melanogaster*. Evolution 60:1602–1611

Schmidt PS, Matzkin L, Ippolito M, Eanes WF (2005a) Geographic variation in diapause incidence, life-history traits, and climatic adaptation in *Drosophila melanogaster*. Evolution 59:1721–1732

Schmidt PS, Paaby AB, Heschel MS (2005b) Genetic variance for diapause expression and associated life histories in *Drosophila melanogaster*. Evolution 59:2616–2625

Tatar M, Chien SA, Priest NK (2001) Negligible senescence during reproductive dormancy in *Drosophila melanogaster*. Am Nat 158:248–258

Tauber MJ, Tauber CA, Masaki S (1986) Seasonal adaptations of insects. Oxford University Press, New York

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

Williams KD, Sokolowski MB (1993) Diapause in *Drosophila melanogaster* females: a genetic analysis. Heredity 71:312–317

Williams KD, Busto M, Suster ML, So AKC, Ben-Shahar Y, Leevers SJ, Sokolowski MB (2006) Natural variation in *Drosophila melanogaster* diapause due to the insulin-regulated PI3-kinase. Proc Natl Acad Sci USA 103:15911–15915