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Further Advances Toward a Model of Gypsy Moth (*Lymantria dispar* (L.)) Egg Phenology: Respiration Rates and Thermal Responsiveness During Diapause, and Age-dependent Developmental Rates in Postdiapause

DAVID R. GRAY*, F. WILLIAM RAVLIN*, JACQUES RÉGNIÈRE†, JESSE A. LOGAN‡

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Measurements of respiration rates were used to support a three-phase model of gypsy moth egg development. Respiration rate was measured on individual eggs that had been reared for 0–155 days at 5°C following diapause initiation. Diapause termination was indicated by the dramatic increase in respiration rate that began approximately 95–125 days after diapause initiation. Respiration rates at 25°C increased each day by approximately 0.0054 μl CO_2/24 h during diapause. After diapause termination respiration rates increased each day approximately 0.1379 μl CO_2/24 h. Eggs were equally responsive to temperature changes during diapause and postdiapause. A 10°C increase in temperature resulted in a 2-fold increase in respiration rate. Developmental rates in postdiapause were found to be age- and temperature-dependent. The initial developmental rate parameters and a temperature-dependent rate change parameter were estimated empirically and a composite function was derived to describe the age- and temperature-dependent behavior of the developmental rate response. Modeled rates increased from 0.0 and 0.078 day^{-1} at postdiapause initiation to 0.01 and 0.20 day^{-1} at physiological age 0.95, at 5 and 30°C respectively.

Lymantria dispar (L.) Diapause termination Postdiapause Thermal responsiveness Development rate

INTRODUCTION

Most existing simulation models of gypsy moth (*Lymantria dispar* (L.)) egg hatch assume that diapause is completed by an arbitrarily chosen calendar date, and that postdiapause proceeds at a rate that is linearly (Johnson *et al.*, 1983) or nonlinearly (Lyons and Lysyk, 1989; Waggoner, 1984) dependent on temperature. These models were fitted to egg hatch data from specific areas in North America and their underlying assumptions can lead to gross errors in simulated egg hatch dates (up to 25 days; personal communication, S. L. Smith, USDA Forest Service, Redding, CA) when applied to locations distant from the areas from which the developmental rate parameter values were obtained.

More recently Tauber *et al.* (1990) proposed a model of gypsy moth egg development encompassing the diapause and postdiapause phases. They hypothesized that the relationship between developmental rate and temperature changes gradually between diapause and postdiapause. Their hypothesis was supported by the data of Masaki (1956) and original data. Sawyer *et al.* (1993) developed a simulation model based on the hypothesis. Their simulation model made no clear distinction between diapause and postdiapause. A single triangular rate function was used to describe the relationship between developmental rate and temperature. Function parameters varied over the course of development. In this way the initial developmental response to temperature has a low temperature threshold and optimum and a low

*Department of Entomology, Price Hall, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0319, U.S.A.
†Forestry Canada, Laurentian Forestry Centre, Sainte-Foy, Quebec G1V 4C7, Canada.
‡USDA, Forest Service, Intermountain Region, Logan, UT 84321, U.S.A.

maximum rate. Over the course of development there is an increase in the temperature threshold and optimum, and the maximum developmental rate. Function parameters were estimated by iterative comparison with previously published egg hatch data from laboratory experiments. The date of onset of diapause was similarly estimated and was assumed to be uniform for all data sets. To date, this model has not been validated with independent data.

Gray *et al.* (1991) interpreted the egg hatch data of Masaki (1956) and M. L. McManus (unpublished) as evidence for a discrete phase transition between diapause and postdiapause occurring after approximately 110 days at 5°C. They proposed a three-phase model of gypsy moth egg development with clear demarcations between the phases, and with each phase being governed by its own developmental rate response to temperature. They described a method of distinguishing phases based on respiration rate and described the temperature-dependent rate of development in prediapause. Further development of this model requires that developmental rate functions be described for the remaining phases.

There is interest in developing a phenological model of gypsy moth egg development for several reasons. A basic necessity of insects living in seasonal climates is the maintenance of an appropriate relationship between life cycle and season. The set of adaptations that promotes appropriate timing of recurring biological events with annual cycles of the environment is the phenology of the species (Tauber *et al.*, 1986). Egg development of the gypsy moth is a critical component of its phenology.

Phenological studies have additional importance when the species involved is of economic importance (Logan *et al.*, 1991), as is the case with gypsy moth egg phenology. Several activities involving gypsy moth management could be aided by an accurate, geographically robust model of phenological events. These activities include application of microbial agents such as *Bacillus thuringiensis* and nucleopolyhedrosis virus over diverse landscapes (Schaub *et al.*, 1995); dispersion of egg masses from "substerilized" gypsy moth adults (Mastro *et al.*, 1989); and conducting detection surveys.

We report here on changes in the pattern of respiration rate of gypsy moth eggs as an indication of the transition between diapause and postdiapause, and on thermal responsiveness from the onset of diapause to egg hatch. We also analyze and model changes in the developmental rate response to temperature that occur during postdiapause.

MATERIALS AND METHODS

Gypsy moth eggs

Gypsy moth (*Lymantria dispar* (L.)) eggs were obtained from a colony of the New Jersey Standard Strain maintained by the U.S. Forest Service, Northeastern Forest Experiment Station (Hamden, CT). Egg masses had experienced 25°C, and a 16 : 8 (light–dark) photoperiod for 42 days. This treatment is designed to ensure completion of the prediapause phase and has been shown to be sufficient for the initiation of diapause (Gray *et al.*, 1991). Egg masses had also experienced a 5°C cold treatment for various lengths of time prior to shipment in order to advance the diapause phase as needed for Experiments 1–3 (see below). Egg masses were packed in a styrofoam cooler with an ice pack and shipped by "overnight express" to our laboratory in Blacksburg, VA. All egg masses continued development in our laboratory as described in the experiments below.

Respiration rate and physiological age

Estimation of thermal responsiveness requires the measurement of the respiration rates of individual gypsy moth eggs. Respiration rates were determined by placing single eggs in individual 1-ml glass autosampler vials (Fisher 03-340-5A; Fisher Scientific, Pittsburgh, PA), each sealed with a rubber septum (Fisher 03-340-13A). Five control vials per temperature treatment were sealed without an egg to obtain estimates of ambient CO_2 concentration. After approximately 24 h a 250 μl gas sample was withdrawn from each vial using a gas-tight syringe (2-0739; Supelco, Belefonte, PA) and injected into an open-system infrared gas analyzer (IRGA) (LI-6251; Li-Cor Inc., Lincoln, NE). A 250 μl sample of 1868 ppm CO_2 (Airco, NJ) was injected as a standard. Gas samples were moved through the IRGA by an aquarium pump. Output signals (mV) from the IRGA in response to the internal temperature of the IRGA and CO_2 concentration were recorded each second by a Campbell CR10 data logger (Campbell Scientific, Logan, UT). The volume of CO_2 in each vial was calculated by comparing the sum of the IRGA CO_2-mediated signals from each vial to the sum of the signals from the standard CO_2 injection. CO_2-mediated signals were corrected for temperature differences. The respiration rate (μl CO_2/24 h) of each egg in each temperature treatment was estimated by subtracting the mean CO_2 volume of the 5 control vials from the calculated CO_2 volume within the egg-containing vial and dividing by the proportion of a 24-h period that the vial was sealed.

Aging is defined by Collatz (1986) as "the unidirectional and irreversible course of intrinsic events which leads the metazoan organism from the beginning to the end of its life". We have adapted this definition to recognize aging within each of three phases of egg ontogeny. In the case of poikilothermic organisms, the rate of the physiological processes that cause aging are temperature-dependent. Therefore, we describe "age" within an ontogenetic phase by time-temperature units, such as x days at y°C, for example. In order to describe age, independent of temperature, we use a unit-free measure of the proportion of the aging process that has occurred. Thus, physiological ages are 0 at the onset of a phase and 1 at completion of a phase, for example. We refer to this measure as "physiological age".

Experiment 1: Thermal responsiveness during diapause and postdiapause phases

Two egg masses in each of 10 age classes experienced 5°C and a 16 : 8 (light–dark) photoperiod for 0, 13, 27, 42, 55, 69, 82, 97, 111 and 125 days prior to shipment from Hamden in order to advance the diapause phase. Immediately following arrival in our laboratory egg masses were placed in a 5°C and a 16 : 8 (light–dark) treatment. Approximately every third day 3 eggs from each egg mass were removed from the 5°C treatment and placed in vials in each of 5, 10, 15, 20 and 25°C treatments. Eggs were allowed to acclimatize for approximately 18 h before vials were sealed. Respiration rates were measured and the eggs were discarded. Respiration rates were measured on 9 days over a 30-day period. Thus, egg mass ages (days at 5°C) ranged from 2 (first measurement on age class 1) to 155 (last measurement on age class 10) days.

Gray *et al.* (1991) defined thermal responsiveness as the linear increase in respiration rate per degree increase in temperature ($\Delta\mu$l CO_2/24 h/°C). We redefine thermal responsiveness here as equivalent to Q_{10}, the familiar description of the increase in reaction velocity caused by a 10°C increase in temperature (see the Discussion for our reasons). The exponential rate function,

$$RS(T) = b\exp(qT), \tag{1}$$

was fitted (PROC NLIN; SAS Institute, 1990) to the respiration rates of each measurement date × egg mass combination. $RS(T)$ is the respiration rate at temperature T, b is a scalar and $q = 0.2303 \cdot \log Q_{10}$. Differences between egg masses in estimates of q and b were examined by a t-test (SAS Institute, 1990). Respiration rates from the 2 egg masses were pooled (see Results) and estimates of q and b were obtained for the pooled data. Relative respiration rates for each measurment date were calculated by dividing each respiration rate by the mean respiration rate of eggs of the same mass at 25°C on the same day. A single Q_{10} value was estimated by fitting equation (1) to the pooled relative rates.

The relationship between time (days at 5°C) and respiration rate at 25°C was examined by linear regression (PROC REG; SAS Institute, 1990). Ranges of egg mass ages were suggested by visual examination of the data (see Results) and the respiration rate vs age relationships were compared between the ranges.

Experiments 2 and 3: Age-dependent postdiapause developmental rates

The abrupt increase in respiration rate at 25°C observed in Experiment 1 (see the Results) suggested that a distinct transition exists between the diapause and postdiapause phases. The continued increase in respiration rate that occurred during postdiapause suggested that the developmental response to temperature may also increase during postdiapause. Therefore, a description of postdiapause development rate that was dependent on both temperature and physiological age was sought. Because the latter cannot be measured directly in postdiapausing gypsy moth eggs, development rate as a function of time was first modeled and then the model was rearranged to replace time with physiological age.

Initial examination of the data (see the Results) indicated that a linear and an exponential description of the increase in developmental rate with time in postdiapause were equally good. The exponential form was selected because it reduced to a simpler function of physiological age. In this model,

$$R_T(t) = R_T(0)\exp(a_T t), \tag{2}$$

R_T is the developmental rate at constant temperature T (°C) at time t, with $t = 0$ being the onset of postdiapause, and a_T describes the amount of change in the developmental rate per unit time at T.

The physiological age in postdiapause after t days is then

$$A_T(t) = \int_0^t R_T(t)\mathrm{d}t = \frac{R_T(0)}{a_T}[\exp(a_T t) - 1]. \tag{3}$$

Developmental rate can be expressed as a time-independent function of physiological age by solving equation (3) for t,

$$t = \frac{1}{a}\ln\left(\frac{a_T A}{R_T(0)} + 1\right), \tag{4}$$

and substituting the result into equation (2):

$$R_T(A) = R_T(0) + a_T A. \tag{5}$$

Experiment 2: Relationship between R_t and t

To determine the relationship between $R_T(t)$ and t, instantaneous developmental rates were estimated in the following manner. Ten egg masses were shipped from Hamden after they had experienced 5°C and a 16 : 8 (light–dark) photoperiod for 96 days. Based upon the results of Experiment 1 (see Results) this treatment was determined to be sufficient for diapause completion in most eggs. Eggs were placed in a 15°C treatment immediately upon arrival from Hamden (day 0) to continue postdiapause development. On days 0, 2, 4 and 6 approximately 25 eggs from each egg mass were placed at each of 4 temperatures (T = 5, 10, 20, and 25°C) for 2–10 days and then returned to 15°C. An additional sample of eggs was transferred from 15°C to 30°C for 2 days on day 0. The remaining eggs from each egg mass (not less than 50 eggs) remained at 15°C. Egg hatch was recorded each day. Because it was not known if developmental rates among egg masses would differ, nor could it be ensured that samples contained an equal number of eggs, egg hatch counts were normalized between egg masses within each temperature × time combination by multiplying each observed number of hatched eggs by $20/n$, where n is the total number hatched from the egg mass in the temperature × time combination. This ensured that each egg mass would contribute equally to the distribution of developmental rates. Twenty was chosen as the normalizing factor, because at

least 20 eggs hatched from most egg masses in each temperature × time combination.

Instantaneous developmental rates at each temperature at $t=0$, 2, 4 and 6 days were estimated (Régnière, 1987) for the 200 normalized observations as

$$R_T(t)=\frac{1-\frac{t_{15}}{t_{\text{med15}}}}{t_T}, \quad (6)$$

where t_{15} is the duration at 15°C, t_{med15} is the median developmental time of the eggs that spent the entire time at 15°C and t_T is the duration at T. The variable t_T was kept as short as possible because as t_T becomes smaller, t_{15} becomes larger, and the small proportion of development that occurred at T is more accurately estimated by $1-t_{15}/t_{\text{med15}}$, regardless of the relationship between $R_T(t)$ and t. Samples were transferred from 15°C to 5°C for 10 days, 10°C for 5 days, 20°C for 2 days and 25°C for 2 days. All treatments had a 16 : 8 (light–dark) photoperiod.

Median values of $R_T(t)$ were estimated from the resulting 200 developmental rates within each temperature × time combination. A saturated model was used in an analysis of covariance (PROC GLM; SAS Institute, 1990) to estimate the effect of T, t and the interaction $T{\cdot}t$ on developmental rate. The effect of T was subsequently removed by dividing each $R_T(t)$ by the mean R_T of the same temperature to create relative developmental rates, $R_T(t)/\bar{R}_T$. Analysis of covariance examined the effect of T, t and $T{\cdot}t$ on $R_T(t)/\bar{R}_T$. A linear and exponential description of the increase in developmental rate with increasing age in postdiapause were compared by examination of the coefficients of determination from the linear and nonlinear fit of $R'(0)+a't$ and $R'(0)\exp(a't)$ to $R_T(t)/\bar{R}_T$, where $R'(0)$ and a' are the temperature-independent descriptions of the developmental rate at the onset of postdiapause and the amount of change in the developmental rate per unit time in postdiapause, respectively.

Developmental rates at 5, 10, 20, 25 and 30°C at $t=0$ provided estimates of $R_T(0)$. A generalized description of $R_T(0)$ was obtained by fitting

$$R_T(0)=\tau+\delta T \quad (7)$$

to these estimates. The parameter δ is the change in developmental rate per degree increase in temperature, and $-\tau/\delta$ is the lower temperature threshold for development. An estimate of $R_{15}(0)$ was obtained by solving equation (7) for $T=15$ in order to provide an additional datum for analysis in Experiment 3.

Experiment 3: Estimation of a_T

Ten egg masses were shipped from Hamden after they had experienced 5°C and a 16 : 8 (light–dark) photoperiod for 96 days. Each egg mass was divided into 6 samples immediately following receipt and samples were reared at 10, 15, 20, 25 or 30°C. Egg hatch was recorded each day. As in Experiment 2, it was not known if developmental rates among egg masses would differ, nor could it be ensured that samples contained an equal number of eggs. Therefore, egg hatch counts were normalized among egg masses by multiplying each observed number of hatched eggs by $100/n$, where n is the total number hatched from the egg mass in the temperature treatment; 100 was chosen as the normalizing factor because at least 100 eggs hatched from most egg masses in most temperature treatments. Median times to hatch were estimated from the resulting 1000 developmental times within each temperature treatment.

Estimates of a_T were obtained by solving equation (3), by iteration, for a_T when $A_T(t)=1$ (hatch), and where t is the median time to hatch at T, and $R_T(0)$ is the median estimate of the developmental rate at postdiapause initiation obtained from Experiment 2.

The relationship between a and T was described with a third degree polynomial:

$$a_T(T)=\omega+\kappa T+\psi T^2+\vartheta T^3. \quad (8)$$

A value of a_5 was not available because gypsy moth eggs do not hatch readily at such low temperatures. However, an increase in the developmental rate at 5°C was observed with increasing physiological age in Experiment 2. To force the model to produce a small, positive value of a at $T=5$°C, the arbitrary datum $a_5=0.01$ was included with the other estimates before fitting equation (8).

Descriptions of developmental rate variability in the population within each temperature treatment were derived by dividing the inverse of each developmental time by the inverse of the median developmental time (M^{-1}) of the same temperature treatment, and then grouping these relative rates into classes of width 0.05. Within each temperature treatment a modified logistic function (Régnière, 1984),

$$F(x)=\{1+\exp[-\gamma(x-1)](0.5^{-\alpha}-1)\}^{-1/\alpha}, \quad (9)$$

was fit to the cumulative probabilities of each class. The cumulative probability of hatch within $x^{-1}{\cdot}M$ days is $1-F(x)$, γ describes the uniformity of the population and α describes the skewness of the distribution. A temperature-independent description of variability was derived by grouping the relative rates from all treatments into classes of width 0.05, and fitting equation (9) to the cumulative probabilities of the classes. Curves were fitted by PROC NLIN (SAS Institute, 1990). Since time to hatch in this experiment is a result of the combined effects of the initial developmental rate $R_T(0)$ and the rate change parameter a_T, these estimates of population variability can be considered estimates of the combined variability in the two factors.

RESULTS

Experiment 1: Thermal responsiveness during diapause and postdiapause phases

There was no significant difference between egg masses in estimates of q or b ($q{:}t=0.0405$, $df=143$, $P=0.97$; $b{:}t=0.9921$, $df=143$, $P=0.32$). Therefore, respir-

ation rates from the two egg masses were pooled. There was no trend to the individual Q_{10} estimates for the 155 days following the onset of diapause [Fig. 1(top)]. Thus, eggs were equally responsive to temperature throughout the experiment and respiration rates at all temperatures were affected similarly by physiological age. A single Q_{10} estimate of 2.15 (SE = 0.031) was obtained from the relative respiration rates.

The effect of age on respiration rate is clearly seen in Fig. 2. Additional time at 5°C caused the respiration rate at 25°C to increase very little for the first 95 days at 5°C (age classes 1–5, first 27 days of class 6 and first 14 days of class 7). Regression analysis estimated that respiration rates increased 0.0054(±0.0004) μl CO_2/24 h per day during this period. After eggs in age classes 6 and 7 experienced 95 days at 5°C, the respiration rate at 25°C increased approximately 0.1379(±0.0209) μl CO_2/24 h per day. We interpreted the dramatic increase in the effect of additional days at 5°C that occurred after day 95 as evidence of a diapause-to-postdiapause phase transition. Similar increases in respiration rate were observed to begin in age classes 8, 9 and 10 from the earliest measurements (after 99, 113 and 127 days at 5°C, respectively) (Fig. 2). This apparent delay in phase transition in age classes 8–10 may be related to the time of shipment of eggs from Hamden (see the Discussion).

Experiment 2: Relationship between r_T and t

A total of 4498 eggs hatched in Experiment 2. Mean egg hatch per temperature × time combination was 250 (SD = 59.1). A minimum of 154 observations occurred in the temperature × time combinations from which we calculated the 200 normalized observations. Egg hatch

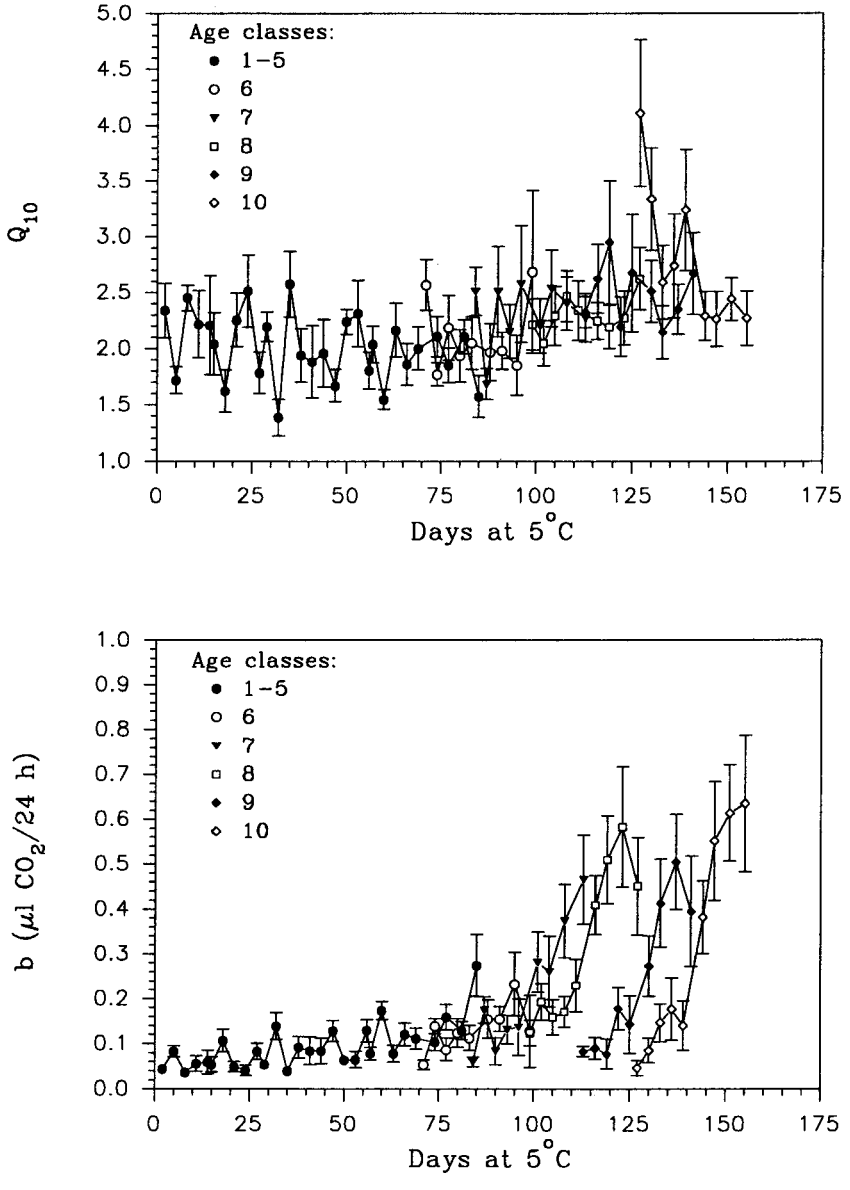

FIGURE 1. The pattern of thermal responsiveness ($Q_{10} \pm$ SE) (top) and the scalar ($b \pm$ SE) of equation (1) (bottom) for 155 days at 5°C following the onset of diapause.

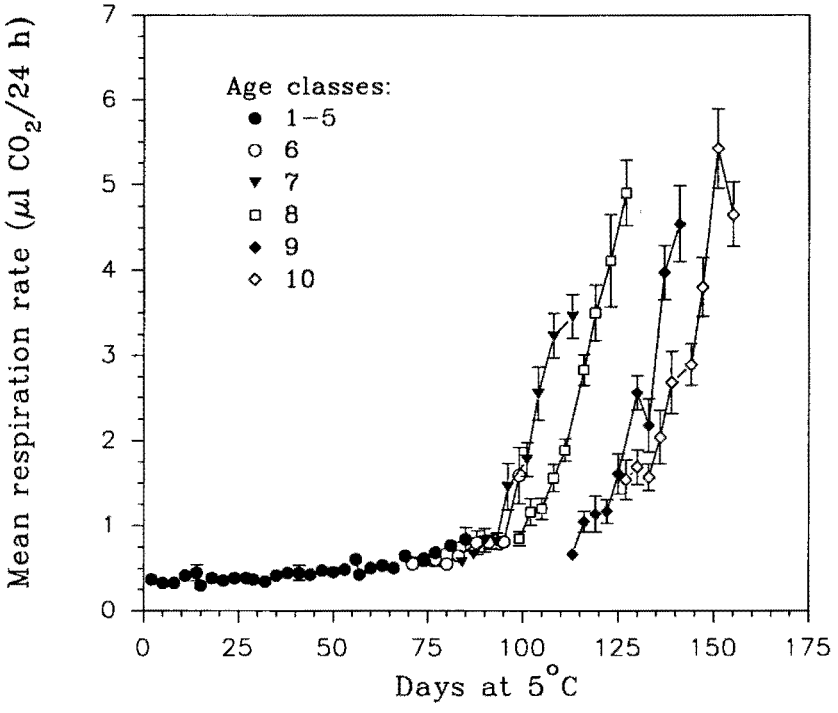

FIGURE 2. The relationship between the mean respiration rate at 25°C (±SE) and time (days at 5°C) for 155 days following the onset of diapause.

began in the 15°C treatment after 6 days, thereby limiting the estimation of $R_T(t)$ to $t=0$, 2, 4 and 6 days.

Analysis of covariance indicated that R_T was significantly affected only by the interaction $T{\cdot}t$ [$F=19.06$, $df=1,12$, $P<0.001$; Fig. 3(top)]. After removing the effect of T by calculating the relative developmental rates $R_T(t)/\bar{R}_T$, the analysis of covariance indicated that there was no effect of T or $T{\cdot}t$, and only t had a significant effect on $R_T(t)/\bar{R}_T$ ($F=15.44$, $df=1,12$, $P<0.002$). These results indicate that the relative change in developmental rate with increasing age (time at 15°C) is the same, regardless of the temperature to which eggs are subsequently exposed [Fig. 3(bottom)]. The relationship between $R_T(t)/\bar{R}_T$ and t was equally well described by the linear model ($R^2=0.793$, $F=53.78$, $df=1,14$, $P<0.001$) or by the exponential model ($R^2=0.800$, $F=56.13$, $df=1,14$, $P<0.001$). Thus, the choice of an exponential over a linear description is amply justified by the ensuing simplicity of equation (5).

A total of 1580 eggs hatched that were transferred from 15°C to the 5 temperature treatments on day 0. Mean egg hatch per temperature treatment was 246 (SD = 42.4). A minimum of 282 observations occurred in the temperature treatment from which we calculated the 200 normalized observations. The median values of the initial developmental rate in postdiapause, $R_T(0)$, were 0.0, 0.021, 0.050, 0.050 and 0.083 days^{-1} at 5, 10, 20, 25 and 30°C, respectively. Equation (7) fitted the median $R_T(0)$ values well ($R^2=0.97$) (Fig. 4). The parameter values are given in Table 1. The initial developmental rate, $R_{15}(0)=0.0319$ days^{-1}, was estimated with equation (7).

Experiment 3: Estimation of a_T

A total of 5760 eggs hatched from the 10 egg masses. Mean egg hatch per temperature treatment was 1152 (SD = 602.5). Estimates of a_T were 0.0948, 0.1971, 0.2611, 0.2023 and 0.0962 days^{-1} for 10, 15, 20, 25 and 30°C, respectively. Equation (8) fitted the estimates of a_T very well ($R^2=0.99$). The parameter values are given in Table 1. The addition of the sixth datum at $T=5$ resulted in positive values for a_T throughout the 5–30°C temperature range (Fig. 5).

The temperature-dependent variability of the population in its developmental rate characteristics [the composite of $R_T(0)$ and a_T] was very well described by equation (9), with all $R^2>0.99$ (Table 1). No discernible pattern was observed in the relationship between temperature and either of the descriptive parameters, thus justifying combining the relative rates from all temperatures. The single, temperature-independent, estimate of population variability also described the observed variability very well ($R^2=0.99$) (Fig. 6). The parameter values are given in Table 1.

DISCUSSION

In conventional experiments investigating developmental rate, sole reliance on measurements of time to complete a life stage is justified by the assumption that developmental response to temperature remains uniform through the stage. However, this assumption is clearly invalid in the case of gypsy moth egg development, as indicated by experiments reported in the literature.

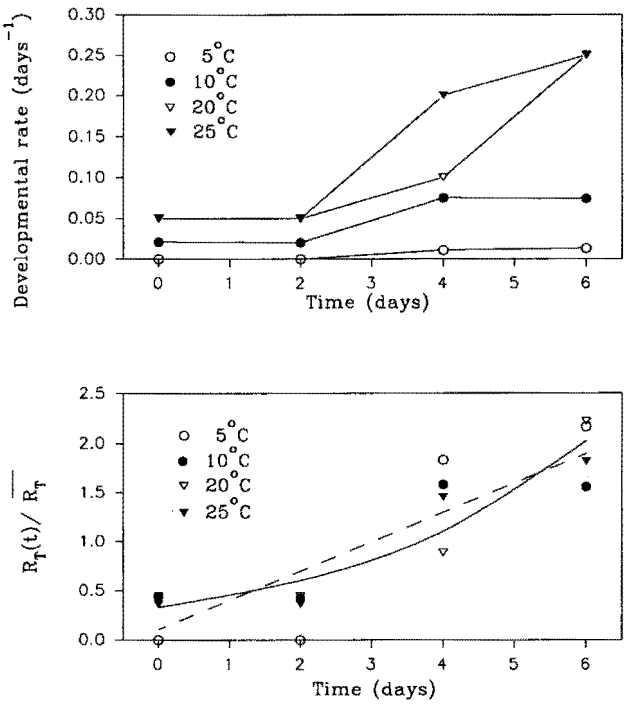

FIGURE 3. The relationship between postdiapause developmental rate and time at 4 temperatures (top). The relationship between observed $R_T(t)/\bar{R}_T$ and time for the same 4 temperatures and the relationship as estimated by a linear (– – –) and an exponential (——) function (bottom). Linear function: $R_T(t)/\bar{R}_T = R'(0) + a't$; $R'(0) = 0.102$, $a' = 0.299$, $R^2 = 0.793$. Exponential function: $R_T(t)/\bar{R}_T = R'(0) \exp(a't)$; $R'(0) = 0.303$, $a' = 0.330$, $R^2 = 0.800$.

Developmental rate is greatest at warm temperatures during early egg ontogeny; diapause requires some period at low temperatures; and egg hatch occurs most quickly under warm temperatures. In order to incorporate this changing developmental response into models of egg development, researchers have divided the egg stage into phases. In the three-phase model of Gray *et al.* (1991)

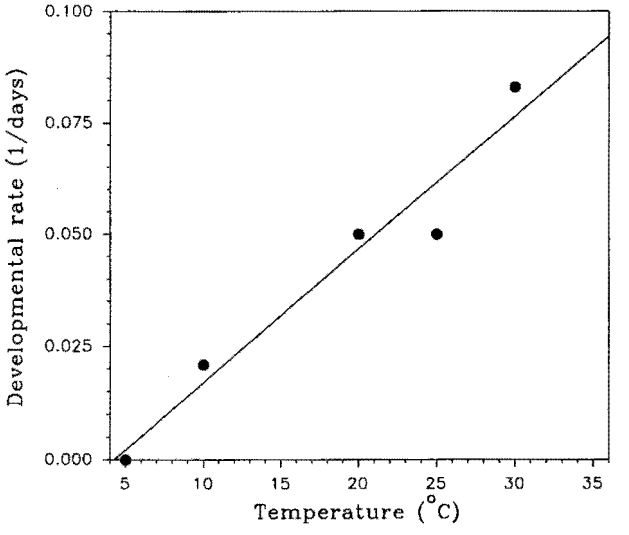

FIGURE 4. The relationship between observed (●) initial postdiapause developmental rate, $R_T(0)$, and temperature, T; and the relationship as estimated by $R_T(0) = \tau + \delta T$ ($\tau = -0.0127$, $\delta = 0.00297$, $R^2 = 0.97$).

each phase is distinct and governed by a unique temperature-dependent developmental rate response, and there is a clear transition between the phases. In the model of Tauber *et al.* (1990), which was further developed by Sawyer *et al.* (1993), there is no clear transition between diapause and postdiapause and the temperature-dependent developmental response increases gradually with increasing physiological age. This gradual change was modeled by subdividing the diapause-to-hatch period into 200 maturity classes. As noted by Sawyer *et al.* (1993), the three-phase model of Gray *et al.* (1991) can be viewed as a special case of their model with only two maturity classes in this developmental period.

The time required for egg hatch from diapause initiation is a result of the sum of the developmental responses governing the developmental process during the entire period. No direct evidence has previously been reported to suggest that developmental response during the diapause-to-hatch period undergoes a distinct transition indicative of two separate phases, or a gradual change indicative of a single phase with a continuum of developmental responses. Estimates of thermal responses during diapause reported by Tauber *et al.* (1990) and Sawyer *et al.* (1993) were inferred from observations of egg hatch. Tauber *et al.* (1990) interpreted the data of Masaki (1956) as evidence of gradually changing thermal responsiveness as diapause progressed, and of an absence of a clear demarcation between diapause and postdiapause. Yet Gray *et al.* (1991) interpreted these same data as evidence of two distinct phases with a clear transition. Modeling the diapause-to-hatch developmental process as two separate phases may be a simplification of a process with gradually increasing responses. On the other hand, subdividing the process into 200 steps with increasing developmental responses may be a way to model a process that has a single response transition when it has not been possible to determine when the transition occurs. The inability to distinguish the developmental phases has been cited as an obstacle to successfully modeling egg development (Lyons and Lysyk, 1989).

Gray *et al.* (1991) proposed measuring thermal responsiveness as an indication of ontogenetic phase. They equated thermal responsiveness with sensitivity to temperature and defined it as the change in respiration rate per degree change in temperature (μl CO_2/24 h/°C). For the following reasons we have redefined thermal responsiveness, here to equal Q_{10}, the familiar description of the increase in reaction velocity caused by a 10°C increase in temperature. A change in physiological state (e.g. from a diapausing to a nondiapausing state) may affect respiration rates in two ways: first, respiration rates may increase by a constant proportion; and second, respiration rates in the nondiapausing state may be more sensitive to changes in temperature. The linear model of thermal responsiveness did not distinguish these effects. Thermal responsiveness (estimated from the linear model) would increase as a result of either a proportionally uniform increase in respiration rate at all temperatures, or a change in sensitivity of the respiration rate to

TABLE 1. Parameter estimates of functions describing temperature-dependent initial developmental rates, and temperature-dependent and temperature-independent variability

Function	τ	δ	ω	κ	ψ	ϑ	γ	α	R^2
Initial rate*	−0.0127	0.00297							0.97
Rate change†			−0.08323	0.01298	0.00099	−0.00004			0.99
Population variability‡									
10°C							15.350	1.014	0.99
15°C							14.206	1.135	0.99
20°C							16.981	1.457	0.99
25°C							10.395	0.959	0.99
30°C							9.221	1.099	0.99
Temp. independent							10.488	0.997	0.99

*$R_T(0)=\tau+\delta T$.
†$a_T(T)=\omega+\kappa T+\psi T^2+\vartheta T^3$.
‡$F(x)=\{1+\exp[-\gamma(x-1)](0.5^{-\alpha}-1)\}^{-1/\alpha}$.

temperature. The linear model also predicts negative respiration rates at moderately low temperatures (approximately 2°C). The concept of Q_{10} is well documented in the literature, and the exponential model of thermal responsiveness distinguishes uniform increases in respiration rate from changes in sensitivity to temperature. Also, the exponential model is more realistic in its predictions that respiration rates will asymptotically approach zero at low temperatures. On the basis of coefficients of determination, the exponential model of thermal responsiveness compared equally well with the linear model ($R^2=0.63$ and 0.64, respectively) within the 5–25°C range examined.

Using the Q_{10} definition it is seen that eggs do not vary in their sensitivity to temperature throughout the diapause and postdiapause phases [Fig. 1(top)]. But a uniformly reduced respiration rate during diapause is indicated by the pattern of the scalar b [Fig. 1(bottom)]. Diapause results in a repressed metabolic rate compared to postdiapause. However, even in this metabolically repressed condition, eggs remain maximally sensitive to temperature changes. The single Q_{10} value estimated from the relative rates (2.15) is in agreement with the range commonly seen for thermochemical (enzymatic) reactions (Hoar, 1975).

However, respiration rates at 25°C strongly suggest that a physiological transition occurred approximately 95 days, at 5°C, after the onset of diapause (Fig. 2). These data clearly display a pattern that can be divided into two phases. During the first phase, the respiration rate at 25°C remains low and differs only very little with age (0–95 days at 5°C) (Fig. 2). An additional day at 5°C during this phase causes the respiration rate at 25°C to increase only 0.0054 μl CO_2/24 h. During the second phase, the respiration rate increases dramatically. An additional day at 5°C causes the respiration rate at 25°C to increase 0.1379 μl CO_2/24 h, approximately 25 times greater than in the previous phase.

The first phase of this pattern is indicative of the diapause state, where diapause has been described by Tauber *et al.* (1986) as a period during which metabolic activity remains low even if current conditions are

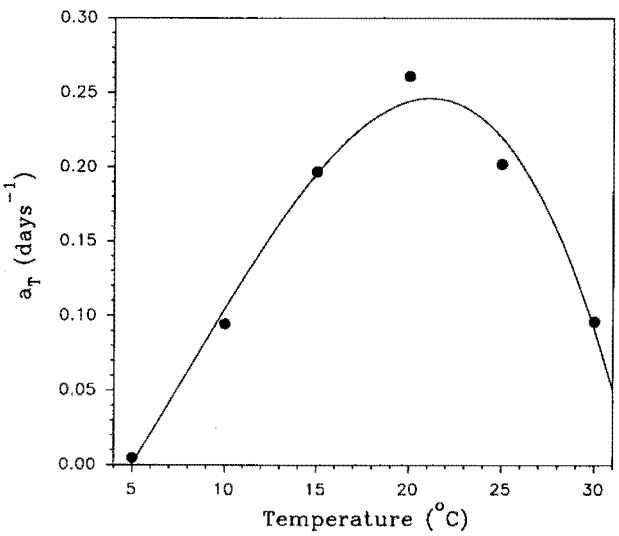

FIGURE 5. The relationship between the observed (●) postdiapause developmental rate change parameter, a_T, and temperature, T; and the relationship as estimated by $a_T(T)=\omega+\kappa T+\psi T^2+\vartheta T^3$; $\omega=-0.08323$, $\kappa=0.01298$, $\psi=0.00099$, $\vartheta=-0.00004$, $R^2=0.99$.

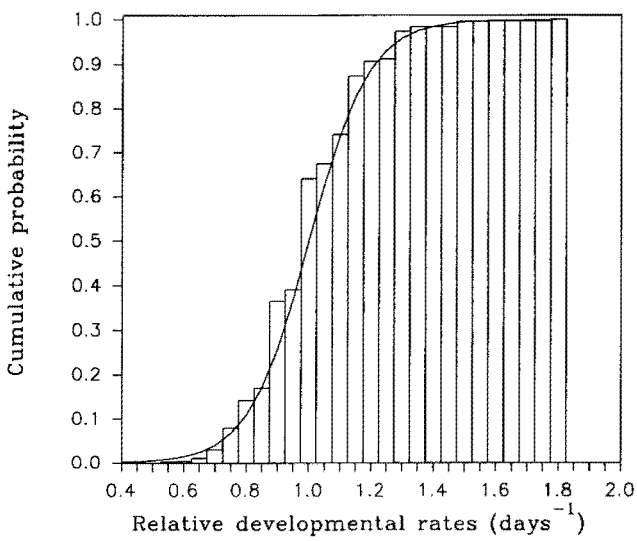

FIGURE 6. Observed cumulative probability of relative developmental rate classes (□) and the variability as estimated by $F(x)=\{1+\exp[-\gamma(x-1)](0.5^{-\alpha}-1)\}^{-1/\alpha}$; $\gamma=10.488$, $\alpha=0.997$, $R^2=0.995$.

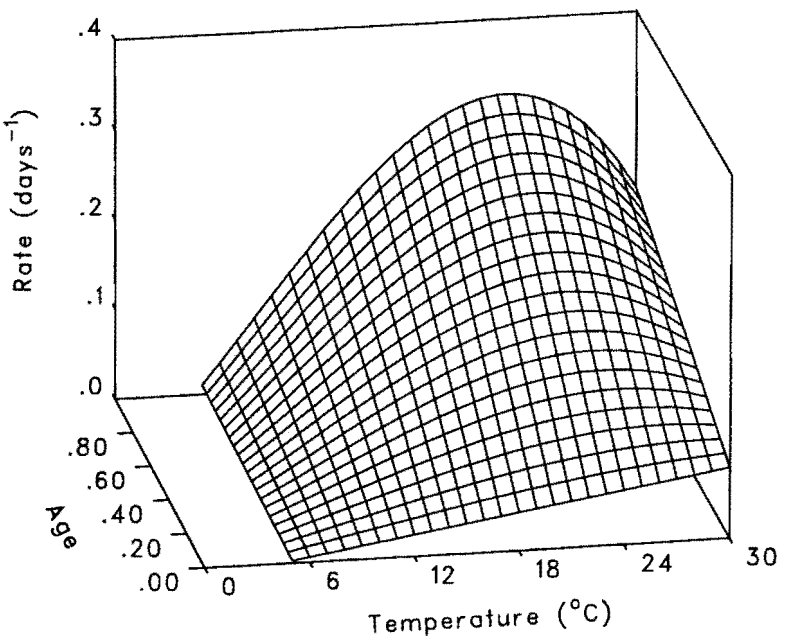

FIGURE 7. The age-dependent relationship between postdiapause developmental rate and temperature.

favorable for development. On this basis we believe that diapause is evident from the low respiration rates observed for the 95 days immediately following diapause initiation (Fig. 2).

The dramatic increase in respiration rate that was first exhibited approximately 95 days after diapause initiation marks the onset of the postdiapause phase. Eggs that exhibited respiration rates greater than 3.0 μl CO_2/24 h, and that were subsequently held at 25°C, hatched within 3 days. Thus, this phase cannot be divided further on the basis of respiration rate. Instead, the respiration rate increases during postdiapause until eggs hatch.

Phase transition was delayed beyond day 95 in age classes 8–10. Although the pattern of increase in the respiration rate in age classes 8–10 is similar to that seen in age classes 6 and 7, the increases began in age classes 8 and 9 only after 99 and 113 days, respectively, at 5°C. An increase may have occurred in age class 10 before the first measurement on day 127. Thus, respiration rates in age classes 8–10 were consistently less than those in the earlier age classes after equal exposure to 5°C (Fig. 2). We are unable to fully explain these differences, but suspect that the attempt to regulate egg mass temperature during shipment was not completely successful. If developmental response to temperature is age-dependent in diapause, as it is in postdiapause, an elevated temperature during shipment would have different effects on diapause development since shipment occurred at different ages in diapause. In any case, the pattern of respiration rate (Fig. 2) strongly suggests that diapause is completed by the combination of a minimum of 95 days at 5°C and the conditions experienced during shipment (age classes 6–9), or by more than 111 days at 5°C (age class 10). Gray *et al.* (1991) hypothesized that phase transition occurred after 111 days at 5°C. Therefore, we are confident that diapause was completed in the eggs of Experiments 2 and 3.

This study provides direct evidence of a clear demarcation between the diapause and postdiapause phases of gypsy moth egg development. Accordingly, a temperature-dependent developmental rate response must be estimated for each phase. Also, the increasing developmental response to temperature with increasing physiological age in postdiapause is more accurately modeled with a temperature- and age-dependent function.

Régnière (1990) described a physiological age- and temperature-dependent developmental rate response in postdiapausing spruce budworm, *Choristoneura fumiferana* (Clem.). In his model the developmental process is sensitive to temperature even at the beginning of postdiapause. However, temperatures of 2.5–8°C had a diminishing effect on developmental rate as physiological age increased and temperatures of 12–24°C had an increasing effect as physiological age increased. Developmental rates were negatively temperature-dependent at young physiological ages and became positively temperature-dependent at more advanced physiological ages. Postdiapause budworm will advance through early physiological ages (approximately 0–0.2) most quickly at low temperatures. Thereafter developmental response is greater under warmer temperatures.

In contrast, this model of gypsy moth postdiapause development describes a physiological age- and temperature-dependent process where developmental rates are positively physiological age-dependent at all temperatures, and positively temperature-dependent at all physiological ages over most of the temperature range examined. However, response is initially slow and relatively insensitive to temperature (Fig. 7). The

developmental response of eggs that are physiologically young in postdiapause is only slightly greater to warm than to cool temperatures. This serves to prevent warm days in late winter or early spring from promoting egg hatch. An equally warm day later in the spring has a greater effect since eggs are more physiologically advanced. For example, the developmental rate at 22°C is 0.05 $days^{-1}$ at postdiapause initiation and 0.25 $days^{-1}$ at physiological age 0.75.

The developmental rate variability of the population was approximately 3 times greater in postdiapause than in prediapause. Eighty percent of the postdiapause population in this study had developmental rates that were between 0.80 and 1.22 times the median (Fig. 6), while 80% of a prediapause population had developmental rates that were between 0.92 and 1.07 times the median (Gray *et al.*, 1991). The slowest and fastest postdiapause developmental rates were approximately 0.4 and 1.8 times the median. We have recorded egg hatch in Virginia that lasts 29 days (unpublished), indicating substantial natural variability.

Using a technique of measuring respiration rates of individual eggs it has been demonstrated here that a clear demarcation exists between the diapause and postdiapause phases. Together with the evidence reported by Gray *et al.* (1991), we conclude that gypsy moth egg development is comprised of three distinct phases: prediapause, diapause and postdiapause. Gray *et al.* (1991) modeled prediapause as a uniform phase with a single developmental rate response. While this may be a simplification of the true process, studies indicate that the three-phase model is insensitive to oviposition date, and to the date of prediapause completion under temperature conditions found over a large geographic range (Gray *et al.*, 1993). Conversely, egg hatch is sensitive to the date of diapause completion. Thus, postdiapause development must be simulated accurately if a model is to be geographically robust. Using empirical estimates of time-dependent developmental times it has been demonstrated that developmental rates in postdiapause are both temperature- and physiological age-dependent. Using estimates of temperature-dependent developmental rate at the initiation of postdiapause, and of temperature-dependent developmental times, a single function was derived to describe the temperature-and physiological age-dependent developmental rates of the postdiapause phase.

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